ISOLATION AND NODULATION TEST OF Rhizobium sp. FROM Pueraria javanica (Benth.) Benth. AND LIABILITY TEST ON THE CARRIER MEDIUM OF PEAT AND COMPOST FROM PALM OIL PALM EMPTY FRUITS

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ABSTRACT

Isolation and Nodulation Rhizobium sp. from Pueraria javanica (Benth.) Benth. and Study of Its Survival on Peat Soil and Compost of Oil Palm Empty Fruit Bunch was done from March to July 2008, in the Laboratory of Plant Biotechnology of Indonesian Oil Palm Research Institute (IOPRI) Marihat Research Station in Pematang Siantar, and Laboratory of Microbiology, Department of Biology, FMIPA USU. The sample of nodules were taken from the roots of Pueraria javanica growing in the oil palm plantation at Sei Aek Fancur, Marihat, and University of North Sumatera. Ten isolates were isolated from the nodules and all of them could form root nodule after 8 weeks of inoculation in a nodulation trial. Another trial was conducted to study the survival of the Rhizobium in 2 different types of media: peat soil and compost of oil palm empty fruit bunch. It was found that all of the isolates could survive in both media during 4 weeks of observation. Statistically, the Rhizobium had a more stable viability in compost.

Keywords: Medium; Compost from Empty Palm Oil; Peat.

INTRODUCTION

One of the conservation efforts of plantation land that is currently widely used is planting ground cover nuts. In Asia, especially the tropics, ground cover legumes are often grown on oil palm and rubber plantations. This plant has various functions, including suppressing weed growth, preventing erosion, thereby reducing the loss of soil nutrients and other organic matter, improving soil structure, restoring soil nutrients and influencing the presence of nitrogen in the soil with nitrogen fixation activity in root nodules (Lehman et al., 1999). With these various functions, planting ground cover legumes is expected to increase the growth of the main crop.

One type of legume that is widely used as ground cover is Pueraria javanica. P. javanica is a type of legume that propagates and is usually used by rubber and oil palm plantations as a pioneer plant that can increase soil fertility. This plant has the ability to bind nitrogen which is needed by
immature main plants (Syamsulbahri, 1996). P. javanica initially grew rather slowly on plantations, but after growing it could last a long time and was more resistant to shade (Riza, 1994).

Rhizobium symbiosis with legume plants is characterized by the formation of root nodules on the plant. Rhizobium has high specificity in forming root nodules. These bacteria will only form root nodules on suitable plants. According to Islami & Utomo (1995), certain species of bacteria are effective for certain types of legumes, but not on other legumes even though they belong to the same group.

The use of Rhizobium as a biological fertilizer has good prospects because it can increase soil productivity, assist the nutrient dissolution process, and increase the carrying capacity of the soil as a result of low microbial activity (Bertham et al., 2005). This symbiotic relationship becomes an alternative source of nitrogen in connection with the increasing use of nitrogen fertilizers in the world (Suharjo, 2001). Planting this ground cover legume at the time of clearing new land or in replanting areas can save about 20-30% of fertilizer (Riza, 1994). Rhizobium associated with legumes is able to meet 80% of the nitrogen requirements of legumes and can increase production between 10-25% (Sutanto, 2002 in Rahmawati, 2005).

The use of Rhizobium as a biofertilizer requires an appropriate carrier medium for its growth. Until now, research is still being carried out to obtain the right carrier medium so as to allow these bacteria to have a high viability in that medium.

The carrier medium that is often used is peat soil. Utilization of peat soil as a carrier medium has several advantages. Apart from having a high moisture holding capacity and high organic matter content which is essential for better shade life of bacterial cultures, peat soil also enhances the survival of Rhizobium cells in the seed coat, especially in dry soil conditions (Rao, 1994).

Another material that can be used as a carrier medium is compost. TKS Compost (Empty Palm Oil Bunches), is compost that is processed from empty oil palm fruit bunches aerobically. Compost that has been cooked contains a variety of important nutrients that plants need. Utilization of the compost as a carrier medium is expected to increase the survival of Rhizobium as well as to increase the function of the compost itself as a biological fertilizer.

The objectives of this study were: 1. To obtain isolates of Rhizobium sp. from P. javanica which is capable of forming root nodules. 2. To determine the viability of Rhizobium sp. of P. javanica on peat soil carrier medium and oil palm empty fruit bunch compost. Benefits of research 1. Develop oil palm plantations by providing new Rhizobium inoculants. 2. To determine the use of Rhizobium from P. javanica as biological fertilizer and to determine the type of carrier medium that is effective and efficient for commercialization purposes. 3. As a reference for further research.

1.1 Ground Cover Plants
There are two types of ground cover crops used in plantations, namely legumes and non-legumes. Ground cover plants from the legume group are commonly used in new or replanted areas, usually in the form of creeping legumes. According to Riza (1994), cover crops have several functions, including reducing soil surface erosion, reorganizing organic matter and nutrient reserves, suppressing weed development, suppressing beetle disturbances, and maintaining soil moisture and improving aeration. In addition, there are advantages to using legumes because root nodules containing Rhizobium bacteria help in fixing free nitrogen from the air. Types of ground cover plants commonly used in oil palm plantations are legumes such as Pueraria javanica, Centrosema pubescens, Calopogonium mucunoides, C. caeruleum, and other species (Syamsulbahri, 1996).

Pueraria javanica (Figure 2.1), is a type of legume that propagates with hard and hairy stems. The growth is fast so that in 5-6 months after planting the closure can reach 90-100% and in the first year it can dominate the plantation area. In addition, this legume is resistant to competing with weeds and can produce a lot of litter, little resistance to shade and drought. Prawirosurokarto et al. (2005).
1.2 Nitrogen Fixing by *Rhizobium*

Approximately 80% of the air in the atmosphere is nitrogen gas (N2). However, N2 cannot be used directly by most organisms. Most organisms use nitrogen in the form of NH3 as a constituent of amino acids, proteins, and nucleic acids. Nitrogen fixation is a process that converts N2 to NH3 which will then be used biologically. This process can occur naturally by microbes (Lindemann & Glover, 1998).

Microbes whose main function is to provide nitrogen by fixing atmospheric nitrogen can be divided into two groups, namely free-living microbes, meaning that they work non-symbiotically or do not have specific associations with certain plants, and microbes that interact with each other. Symbiotic with certain plants (Yuwono, 2006). One example that is currently being studied is the symbiotic relationship between Rhizobium and legumes.

Rhizobium is a gram negative bacterium, aerobic, does not form spores, rod-shaped with a size of about 0.5-0.9 m. This bacterium belongs to the Rhizobiaceae family. These bacteria are widely found in the root area (rhizosphere) of legumes and form a symbiotic relationship with a special host (Yuwono, 2006).

Rhizobium is a facultative symbiont, can live as a normal component of the soil microflora in the absence of a host plant, but still live freely as a heterotroph depending on the presence of host plant roots. The population of Rhizobium in the rhizosphere of ordinary legumes reaches 106 cells/gram or more (Richards, 1987). In soil, these bacteria are free-living and motile, obtaining nutrients from the remains of dead organisms. Free-living Rhizobium cannot fix nitrogen and has a different shape from other bacteria found in plant root nodules (Burdas, 2002).

1.3 Nodulation Specificity *Rhizobium*

Rhizobium bacteria can only symbiotically with legume plants by infecting their roots and forming root nodules in them (Rao, 1994). In many cases, Rhizobium indigenous inoculants are sometimes ineffective on introduced plants (Richards, 1987). The principle of cross-inoculation grouping is based on the ability of Rhizobium isolates to form root nodules in a limited genus of closely related legume species. All Rhizobium that can form root nodules on the roots of certain types of legumes are collectively included in one species (Rao, 1994). Some degree of specificity in nodules and legumes can be arranged in groups, members of one group usually form nodules with a given legume but their ability to fix N is a function of both the host plant and the bacteria themselves (Richards, 1987).

Not all types of legumes tested so far have formed nodules, about 10% of the types have been examined. The genus Rhizobium which belongs to the family Rhizobiaceae consists of several species of legumes but not others. R. leguminosarum, for example, was able to form effective nodules on the roots of Pisum sativum, Vicia and Lithyrus, but not on Trifolium, Medicago sativa and many other legumes. R. trifolii formed nodules on various types of clover but not on Pisum sativum, bean and
others (Table 2.1). Groups of different plant species that may be nodules with the same Rhizobium species are called cross-inoculation groups (Mulder & Woldendorp, 1969). Several species of Rhizobium and their symbiotic plants (Rao, 1994):

| Rhizobium spp. | Cross inoculation group | Legume type |
|----------------|-------------------------|-------------|
| R. leguminosorum | Pea group Peanut group Clover | Pisum, Vicia, Lens Phaseolus Trifolium |
| R. phaseoli | group Alfalfa group Lupini group | Medicago, Melliotus, Trigonella |
| R. trifolii | Soy group | Lupinus, Ornithopus |
| R. Melioti | Cowpea group | Glycine |
| R. lupini | R. japonicum | Vigna, Arachis |
| Rhizobium sp. | |

1.4 **Mechanism of Nodule Formation**

Rhizobium symbiosis with legumes is characterized by the formation of root nodules on the host plant (Figure 2.2). The formation of root nodules begins with the secretion of plant metabolic products into the root area (nod factors) which stimulate bacterial growth, in the form of liposaccharides (Burdas, 2002). The root exudates produced by these legumes have a beneficial effect on the division of Rhizobium in the soil (Mulder & Woldendorp, 1969).

1.5 **Nitrogen Fixing Mechanism in Root Nodules**

The main role of Rhizobium is to fix nitrogen in the presence of nitrogenase activity. The level of nitrogenase activity determines the amount of ammonium that Rhizobium gives to plants (Martani & Margino, 2005). Rhizobium nitrogenase activity is determined by 2 types of enzymes, namely dinitrogenase reductase and dinitrogenase enzymes. Dinitrogenase reductase with Fe protein co-factor acts as an electron acceptor to be further forwarded to MoFe protein, while dinitrogenase enzyme which has MoFe protein plays a role in N2 binding (Hughes, 1996 in Martani & Margino, 2005).

Richards (1964) simplify the nitrogen fixing reaction in legume root nodules in the following equation:

\[
N_2 + 8H^+ + 8e^- + 16Mg-ATP \rightarrow 2NH_3 + H_2 + 16Mg-ADP + 16Pi
\]

According to Arimurti (2000), the ability of Rhizobium to fix nitrogen from the air is influenced by the size of the root nodules and the number of root nodules. The larger the root nodule or the more nodules formed, the more nitrogen is fixed. The more active the nitrogenase, the more nitrogen supply for plants, so it can improve plant growth. Martani & Margino, 2005). The amount of N2 that can be fixed by legumes varies widely, depending on the type of legume, cultivar, type of bacteria and where the bacteria grow and especially soil pH (Islami & Utomo, 1995).

1.6 **Utilization of Rhizobium as Biofertilizer**

Land planted with legumes sometimes still requires additional inoculation of Rhizobium. However, inoculated plants may not always be able to compete well with natural soil microbes or against soil conditions that are less favorable for growth of the added strain. Ladha et al., 1988). The presence of ineffective natural microbes in large numbers can interfere with successful inoculation practices. In unfavorable conditions, such as in acid soil areas in Sumatra, the number of natural Rhizobium is lower or not present at all (Waluyo et al., 2005).

Empty Palm Oil Bunch Compost (TKS) is compost made from empty palm oil bunches which are chopped and then doused with liquid palm oil waste and left for some time. The composting process itself is aerobic and does not require additional microorganisms from outside (Ispandi & Munip, 2005). Cooked compost has a C/N ratio of 15 (Table 2.2) with an effective standard C/N ratio...
ranging from 30:1 to 40:1. The nutrient content of the compost can also be enriched with certain elements according to the needs of the plant and is expected to increase the survival of Rhizobium. Nutrient content of oil palm empty fruit bunch compost (Darnoko & Sutarta, 2006):

| Description | TKS (P) | Compost (P) |
|-------------|---------|-------------|
| P (%)       | 0.068   | 0.022       |
| K (%)       | 2.18    | 3.45        |
| Ca (%)      | 0.4     | 0.72        |
| Mg (%)      | 0.13    | 0.54        |
| C (%)       | 48.44   | 29.76       |
| N(%)        | 0.74    | 1.98        |
| C/N         | 64.46   | 15.03       |
| Water       | 69.96   | 54.39       |

### RESEARCH METHOD

#### 2.1 Materials and tools

The sample used to obtain Rhizobium isolates was the root nodule of *P. javanica*. Yeast Mannitol Agar + Congo Red media and Yeast Mannitol Broth media were used as isolation and propagation media for Rhizobium isolates. The nodulation test used mineral soil around the Marihat Palm Oil Research Center area of Pematang Siantar as a planting medium. Meanwhile, for the viability test using peat soil and oil palm empty fruit bunch compost as the carrier medium.

The tools used include autoclaves, vials, spatulas, petri dishes, pipettes, loops, hockey sticks, test tubes, shakers, polybags, plastic containers, and vortexes.

#### 2.2 Sample Collection

Sampling was carried out at the Sei Aek Pancur Oil Palm Estate on March 12 and April 4, 2008, at the Marihat Palm Oil Estate on April 16-18, 2008, and the oil palm plantation area around the University of North Sumatra (USU) on April 14-15, 2008. Sampling was carried out at 5 points for each location.

P. javanica which has root nodules removed, then put in a plastic bag. The samples obtained were taken to the Biotechnology Laboratory of Marihat Palm Oil Research Center Pematang Siantar for further processing.

#### 2.3 Isolation of *Rhizobium* from Nodules

Root nodules are selected that are well shaped, pink and healthy. These root nodules are then surface sterilized. The root nodules were immersed in a vial containing sterile distilled water for 5 minutes. Sterilization was continued by immersing it in 96% alcohol for 10 seconds. Then soaked in 5.25% chlorox solution for 10 seconds and rinsed with sterile distilled water 3-4 times. A total of 6 sterile root nodules were put into a sterile petri dish, squeezed using a spatula, then 1 ml of sterile distilled water was added. This suspension was then isolated on Yeast Mannitol Agar + Congo Red media using the spread plate method, incubated at 28°C for 24-48 hours. On YMA + Congo Red media, Rhizobium colonies were white, transparent, circular, convex, and slightly slimy.

#### 2.4 Characterization and Propagation of *Rhizobium* sp.

Isolates grown on YMA + Congo Red media were characterized by morphology and Gram staining. The obtained Rhizobium isolates were then propagated on YMA + Congo Red media.

#### 2.5 Nodulation Test of *Rhizobium* sp. Against *Pueraria javanica*

The nodulation test was carried out using mineral soil obtained around the Biotechnology Laboratory of PPKS Marihat Pematang Siantar as a planting medium. The soil was first sterilized for 3x30 minutes with an interval of 24 hours at a temperature of 121°C and a pressure of 15psi. A total of 1 ose was taken from Rhizobium sp. isolate, put into a test tube containing 10 ml of Yeast Mannitol
Broth (YMB) media, then shaken for 12 hours at 150 rpm. These bacterial cultures are then inoculated into polybags containing sterilized soil. Pueraria shoots were taken, then surface sterilized with chlorox solution and rinsed with sterile distilled water. Then the shoots were planted in the inoculated planting medium. The repetition was done 3 times. At week 8 observed and counted root nodules formed.

2.6 Rhizobium sp. Viability Test on the Carrier Medium

Rhizobium isolates capable of forming root nodules were then tested for viability on a carrier medium. The carrier medium used was peat soil and compost of oil palm empty fruit bunches. The peat soil used is raw peat soil obtained from PT Asam Jawa, Kota Pinang. Meanwhile, the compost for empty fruit bunches was obtained from Sei Aek Pancur, Tanjung Morawa. All carrier medium was sterilized beforehand. Sterilization was carried out at 121°C, 15 psi for 3x30 minutes with an interval of 24 hours. The pH of the carrier medium was measured, then added with CaCO₃ until the pH reached neutral (pH=7). Peat soil has a pH of around 5.6, while compost has a neutral pH. For peat soil, CaCO₃ is added so that the pH is neutral. The carrier medium was then divided into plastic containers of 100 grams for each container. A total of one ose of isolate was mixed with 10 ml of YMB and then shaken for 12 hours at a speed of 150 rpm. Then inoculated evenly into each carrier medium. Each was carried out 3 repetitions.

The number of colonies was counted from week 0 to week 4 using the Standard Plate Count (SPC) method on YMA + Congo Red media. The carrier medium was filtered as much as one gram using a 1x1 mm sieve and then put into a test tube, then added with sterile distilled water to 10 ml, then homogenized with a vortex. Then it is left for 30 seconds for the soil particles to settle. The dilution was carried out to 10⁻⁶. A total of 0.1 ml was taken from the last tube dilution taken using a micro pipette and then poured into a test tube containing 5 ml of sterile YMA + Congo Red medium, homogenized with a vortex, poured into a sterile petri. The culture was incubated at 28°C for 24 hours. The number of colonies that grew was then counted. The number of living cells is calculated by the formula:

\[ \text{CFU/g of carrier medium} = \text{Number of visible colonies} \times \text{df (dilution factor)} \]

2.7 Experiment Design and Data Analysis

The Rhizobium viability test on the carrier medium used a Factorial Completely Randomized Design (CRD) with 3 replications. This design consists of 2 factors:

a. Factors of origin of isolates, consisting of:
   - Aek Pancur 1 (AP1)
   - Aek Pancur 2 (AP2)
   - Aek Pancur 3 (AP3)
   - Aek Pancur 4 (AP4)
   - Marihat 1 (M1)
   - Marihat 2 (M2)
   - Marihat 3 (M3)
   - Marihat 4 (M4)
   - USU 1 (U1)
   - USU 2 (U2)

b. Incubation time factor (T), consisting of:
   - Week 0 (T0)
   - Week 1 (T1)
   - Week 2 (T2)
   - Week 3 (T3)
   - Week 4 (T4)
The missing data was recovered by using the missing data method with iteration techniques. Then the data was transformed on a logarithmic scale, then analyzed using Analysis of Variance (ANOVA), followed by the Duncan New Multiple Range Test (DNMRT) mean test.

RESULTS AND DISCUSSIONS

3.1 Isolation of Rhizobium from Nodules of P. javanica

Samples of root nodules of P. javanica were obtained from 3 locations of oil palm plantations, namely Sei Aek Pancur oil palm plantations, Marihat oil palm plantations, and oil palm plantations at the University of North Sumatra. These three locations have a soil temperature of around 27°C, and a soil pH of 6-6.9. Sei Aek Pancur and USU plantation locations have dry and hard soil textures, while Marihat plantations have sandy soil textures. These three sites had never been inoculated with Rhizobium before. From the isolation results, 10 Rhizobium isolates were obtained with the following characteristics:

Table 4.1 Colony characteristics of Rhizobium isolates

| Location  | Code Isolate | Colony morphology | Coloring gram Shape | Type |
|-----------|--------------|--------------------|---------------------|------|
| Sei Aek   | AP1           | Circular, convex, flat edge, slimy, White color. | stem | Negative |
|           | AP2           | Circular, convex, flat edge, slimy, White color. | stem | Negative |
|           | AP3           | Circular, convex, flat edge, slimy, White color. | stem | Negative |
|           | AP4           | Circular, convex, flat edge, slimy, White color. | stem | Negative |
| Marihat   | M1            | Circular, convex, flat edge, slimy, White color. | stem | Negative |
|           | M2            | Circular, convex, flat edge, slimy, White color. | stem | Negative |
|           | M3            | Circular, convex, flat edge, slimy, White color. | stem | Negative |
|           | M4            | Circular, convex, flat edge, slimy, White color. | stem | Negative |
| USU       | U1            | Circular, convex, flat edge, slimy, White color. | stem | Negative |
|           | U2            | Circular, convex, flat edge, slimy, white color. | stem | Negative |

From the table above, it can be seen that the isolates obtained had almost the same colony characteristics, namely circular, convex, flat edges, white and slimy. On microscopic observation with Gram staining, the bacterial cells obtained were rod-shaped with Gram negative type. These characteristics are in accordance with the statement of Rao (1994), that on agar media with the addition of Congo Red, Rhizobium will form clear white colonies, shiny, prominent, with intact overall edges, and has watery characteristics (Arimurti et al., 1983). Rhizobium is a Gram negative bacterium, aerobic, and does not form spores, rod-shaped with a size of about 0.5-0.9 m (Yuwono, 2006).
3.2 Nodulation Test of Rhizobium sp. Against P. javanica

The ten isolated isolates were then tested for their ability to form root nodules. The nodulation test was carried out on the planting medium using mineral soil obtained from the area around the Marihat PPKS. Sandy soil type with a pH of 6.7-7, and a temperature of about 28°C. From this test, it was found that all isolates were proven to have the ability to form root nodules on P. javanica (Table 4.2). This indicated that the Rhizobium isolate obtained was an effective species on P. javanica.

The isolate that formed the most root nodules was Rhizobium isolate with isolate code M3, which was 39 nodules per test plant (Table 4.2). This may be due to the isolate being a natural isolate in the area, causing this isolate to adapt more quickly to the growing medium used so that the process of forming root nodules is faster and more nodules are formed. This trend can be observed from the average number of root nodules formed by Marihat isolates, which was more than isolates from other locations, which was around 30.7 root nodules per plant.

| Isolation Code | Number of root nodules | Total | Average | Averagebintilakar based on the origin of the isolate |
|----------------|------------------------|-------|---------|-----------------------------------------------------|
| AP1            | 0                      | 18    | 50      | 68                                                 | 22.7 |
| AP2            | 5                      | 50    | 6       | 61                                                 | 20.3 |
| AP3            | 32                     | 14    | 36      | 82                                                 | 27.3 |
| AP4            | 25                     | 36    | 20      | 81                                                 | 27   |
| M1             | 8                      | 50    | 48      | 106                                                | 35.3 |
| M2             | 0                      | 24    | 26      | 50                                                 | 16.7 |
| M3             | 71                     | 0     | 46      | 117                                                | 39   |
| M4             | 0                      | 85    | 10      | 95                                                 | 31.7 |
| U1             | 0                      | 8     | 3       | 11                                                 | 3.7  |
| U2             | 48                     | 16    | 40      | 104                                                | 34.7 |

Table 4.2 Number of root nodules from the nodulation test
The average number of root nodules from Sei Aek Pancur isolates was about 24.3 nodules, and the lowest was USU isolates with an average of about 19.2 nodules per plant. This indicates the influence of environmental factors, especially the condition of the growing media on the adaptation process of Rhizobium and the formation of root nodules in the tested plants. The isolates obtained from Sei Aek Pancur and USU were not natural isolates in the soil used, causing these bacteria to take longer to adapt and form root nodules. According to Shantharam and Matto (1997), the Rhizobium strain to be used should be isolated from a certain area and inoculated back into the same environment to ensure successful inoculation.

From the results obtained, the root nodules formed are root nodules that are effective in nitrogen fixation. This can be observed from the reddish color that appears on the root nodules (Figure 4.2). According to Richards (1987), the efficiency and effectiveness of a Rhizobium strain on root nodules can be observed from the reddish color due to the presence of leghaemoglobin pigment contained in it. Because it does not have leghaemoglobin, so nitrogen fixation cannot occur in the root nodules (Yuwono, 2006).

The leghaemoglobin in root nodules is located between the bacteroids and the membrane sheath that surrounds them. The amount of this pigment in root nodules has a direct relationship with the amount of nitrogen fixed (Rahmawati, 2005). Leghaemoglobin is only found in healthy root nodules, whereas unhealthy plants have white root nodules.

**3.3 Rhizobium sp. Viability Test on the Carrier Medium**

The ten Rhizobium isolates obtained had good viability on peat soil carrier medium and oil palm empty fruit bunch compost. The number of live cells at week 4 was about 10^8-10^9 cells/g of carrier medium. This amount is in accordance with the minimum standard for the number of Rhizobium inoculant cells, which is 10^8-10^9 cells/g of carrier medium (Grahamweiss, et al., 1987). The ten Rhizobium isolates tested showed different viability on the two types of carrier medium used. Tuzimura et al. (1966) in his research stated that the population of Rhizobium was influenced in a complex manner by the type of soil and the type and strain of the bacteria itself. According
to Suprapto (1999) There are several factors that affect the growth of Rhizobium, including soil pH, temperature, light, and nutrients. The optimum reaction for the growth and development of Rhizobium at pH 5.5-7.0 (Elfiati, et al., 2006) and temperatures between 28-31°C (Zahran, 1999).

On peat soils (Figure 4.3), the population of several isolates such as AP1, AP2, AP3, AP4, M1, M2, M3, and M4, decreased from week 1 to week 2, the number then began to increase in 3rd week and 4th week. However, in some isolates such as isolates U1 and U2, the population tended to be stable in the first week of storage, then decreased in the 4th week. Based on data analysis (Appendix 6, p. 41), statistically the time factor had a significant effect on the population of Rhizobium at week 1 to week 3, but the number increased again at week 4. As in isolate M3, which formed more root nodules in the nodulation test, there was an increase in the isolate population from the beginning of inoculation, which was 8.85 (9.4 x 10^7 cells/g carrier medium) to 8.89 (1.017 x 10^7 cells/g carrier medium) at week 4 (Appendix 7, p. 42). A significant decrease in the number of cells was observed in M4 isolates, especially at week 2, which was 7.53 (4.7 x 10^7 cells/g carrier medium) from the initial number of 8.4 (3.5 x 10^7 cells/g carrier medium). The number then increased again to 8.7 (63.3 x 10^7 cells/g of carrier medium) at the 4th week. This may be due to the difficulty of the isolates adapting to the raw peat soil used. According to Riwandi (2002), the main component of tropical peat soil which is dominated by lignin is about 75%, making it difficult to decompose. Raw peat (fibric) is a type of peat that is composed of more than 2/3 of coarse organic matter. This soil has a high water holding capacity, but the nutrients are still in organic form and are not available. According to Sagiman (2006), raw peat has a C/N ratio exceeding 30, this indicates a lack of nitrogen, even though the total N analysis results show a high number. At this stage, some cells may not be able to adapt and die. Cells that are able to survive and utilize other organic materials as a source of nutrition then experience growth so that the number increases again in the 3rd and 4th weeks.

In this study, although there was a significant decline in the population at the beginning of inoculation, the isolates tested had the ability to survive on the carrier medium for 4 weeks. Based on research conducted by Syamsulbahri (1997), peat medium is a good carrier medium as a place for Rhizobium to survive longer. Peat carrier medium is a medium containing more than 90% organic matter. In addition, peat has a high moisture holding capacity and has a large number of pores for oxygen exchange. (Rao, 1994).

The adjusted condition of the peat medium also supports the growth of these bacteria. According to Kremer & Peterson (1983), peat can retain high levels of Rhizobium (> 10^8 per g) when incubated at 3-28°C. The addition of CaCO3 causes the pH of the peat soil to increase, thereby increasing the neutrality of the isolate’s viability in it. Sutarto (1994) in his research stated that liming treatment on acid soil can improve soil pH so that it stimulates the formation of more nodules and maintains Rhizobium so that they can live normally.

Figure 4. Viability curve of 10 Rhizobium isolates from P. javanica on peat soil carrier
Figure 5. Viability curve of 10 Rhizobium isolates from P. javanica on oil palm empty fruit bunch compost carrier medium

The ten Rhizobium isolates tested had a more stable viability in compost than in peat soil. Based on the results of data analysis (Appendix 9, p. 48), statistically the storage time factor significantly affected the number of bacterial populations in the 1st week. Based on the curve (Figure 4.4), the population of all isolates tended to increase after the 1st week. This may be because compost is the result of the decomposition of organic materials, so that the nutrients contained in it are more available and in a simpler form, and it is easier for these bacteria to use for growth. Oil palm empty fruit bunches are composed of 45-50% cellulose, and about 25-35% hemicellulose and lignin (Schuchardt, et al. 2002). After processing, the compost contains about 48.44% C; N 0.74%; Ca 0.4%; K 2.18%; P 0.068%; and Mg about 0.13%. Cooking compost has a C/N ratio of 15 with a standard effective C/N ratio ranging from 30:1 to 40:1 (Darnoko & Sutarta, 2006). The high content of nutrients in this compost is able to support the survival of Rhizobium in it.

The difference in the humidity of this compost medium with peat soil does not seem to have a significant effect on the Rhizobium population in the first week of storage. Even in AP3, M2, and M4 isolates, the population continued to increase until the 4th week. However, in some isolates such as AP1, AP2, AP4, M3, U1 and U2, there was a decline in the population at week 3 (Figure 4.4). In AP2 isolates, the total population decreased significantly from 8.9 (138.7 x 107 cells/g carrier medium) at week 3 to 8.4 (33.7 x 107 cells/g carrier medium) at week 3.4 (Appendix 10, p. 49). Likewise for M3 isolates, there was a decrease in the population of the isolates from 8.97 (95.3 x 107 cells/g carrier medium) to 8.47 (30.7 x 107 cells/g carrier medium) in the 4th week. This may be due to the low moisture content in the compost. causing a gradual decrease in the population of these isolates on the carrier medium used. According to Alexander (1961), the population of Rhizobium will not decrease immediately when added to the soil in large quantities, although the average survival will decrease with increasing temperature.

From the results obtained in the viability test, it was shown that the two types of carrier medium used were able to support the viability of Rhizobium. The availability and handling of peat soils, which are relatively difficult in some areas, may be overcome by using alternative carrier media such as compost, which is more readily available. Composting is quite simple and utilizing waste from oil palm is one of the advantages, in addition to reducing handling costs while increasing its function as biological fertilizer.

From this study, isolate M3 was the isolate that had the most potential in forming root nodules. However, the application of Rhizobium inoculum to plantations sometimes requires more than one Rhizobium strain. According to Yuwono (2006), biological fertilizers can be made using more than one type of microorganism, or from one type of microorganism but with different strains. Waluyo, et al. (2005), in his research succeeded in isolating about 27 Rhizobium strains from Java and 24 Rhizobium strains from Sumatra which form root nodules on soybean plants. This shows that the Rhizobium that forms root nodules in a plant species is not only one strain but consists of many different strains. In addition, the possibility of competition from these isolates with other natural soil
bacteria during inoculation can be an obstacle to the success of inoculation, in addition to the effectiveness of the Rhizobium strain used. For this reason, it is recommended to inoculate Rhizobium with various different effective strains to ensure the success of inoculation on plantation land.

CONCLUSION

From the results obtained it can be concluded:
1. From the isolation carried out on the root nodules of Pueraria javanica, 10 isolates of Rhizobium were obtained which have the ability to form root nodules on these plants. The isolate that formed the most nodules was M3 isolate obtained from the Marihat Oil Palm Plantation.
2. The ten Rhizobium isolates obtained had a more stable viability on the carrier medium of oil palm empty fruit bunches.

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