

Original Research Article

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Utilization of Phosphate Solubilizing Rhizobacterium Derived from Leguminosae Plants to Stimulating Plant Growth and Induce Systemic Resistance of Peanuts (*Arachis hypogaea* L) to Plant Diseases

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ABSTRACT

Keywords

Phosphate solubilizing rhizobacterium, Root of leguminosae, Inducer of systemic resistance against plant pathogen, Active Sand formulation, Bio fertilizer

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In general, the compounds needed to improve the formation of hair roots are the growth hormone Indol Acetic Acid (IAA), this hormone is in addition produced by Plant Growth-Promoting Rhizobacteria (PGPR). These bacteria, although applied at the root, are also capable of improving other parts of the plant to produce toxic compounds for pests and diseases, so plants resistant to pests, the bacteria are also called Systemic Acquired Resistance (SAR) bacteria or Induced Systemic Resistance to pests. From the results of this research, it was found that the Phosphate Solubilizing Rhizobacterium, that have been formulated in the form of biofertilizer formulation of, Active Sand Formulation, and Compost Formulation, able to improve plant growth in the form of plant height, number of leaves and number of branches and Yield compared to control. Liquid and flour formulations, however, generally very low stimulating plant growth. The kind of Phosphate Solubilizing Rhizobacterium capable to improve the growth and yield of peanut plants are *Serratia marcescens*, *Enterobacter cloacae* and *Achromobacter spanius*. Peanut crops that are applied with Biofertilizer Phosphate Solubilizing Rhizobacterium, less able to protect the plant from the infection of leafspot (*Cercospora arachidicola*), leaf spots (*Alternaria arachidis*) and leaf blight (*Leptosphaerulina crassiasca*). Peanut crops that are applied with biofertilizer Phosphate Solubilizing Rhizobacterium, able to protect peanut plants from rust disease (*Puccinia arachidis*). The best formulation biofertilizer of Phosphate Solubilizing Rhizobacterium is an Active Sand formulation.

Introduction

In Indonesia, peanuts are one of the important sources of the plant is rich in protein. Peanut consumption that was ingredients processed in various forms of food such as cakes, snacks, or other processed products. In developed countries peanuts are also a source of oil plant (Adisarwanto, 2000). In

Indonesia, most peanuts are cultivated in paddy field in dry season, peanuts can be planted on light textured or heavy soil, that can absorb water well, so there is no puddle. However, the most suitable soil is the lightly textured soil of good drainage, crumbs and loose. Peanuts can also produce in clay soils, although high risk, that is dead by flooded, and the soil is difficult to remove from the

Pods and many pods are left in the soil during harvest.

Production of Indonesian peanut from 2007 to 2010 showed fluctuation, and in 2011 decreased 13.11% compared to 2010. As a result, Indonesia had to import peanuts from other countries such as Vietnam, China, Thailand, India, and Australia, (Dinarto and Asrani, 2012). Peanut crop yields in Indonesia are low, because they are still below production potential. The result of local peanuts only reached 1.45 tons / ha, lower than the potential yield of superior varieties such as; Panther and Lions varieties that can reach 4.5 ton / ha (Adisarwanto, 2000).

In addition, the low production of peanuts in Indonesia is caused by pests and diseases and plants are less get maintenance by farmers, and usually planted peanuts in the fields after rice harvested and soil tillage is not good. In addition peanut plants are planted in paddy fields, sensitive to disease infection because plants lack enough nutrients to produce secondary metabolites that can protect plants from the disease pathogens and insect pests (Hidayat and Mulyani, 2002)

While the use of synthetic pesticides causes microbial development disturbed in soil and nutrient balance in the soil disturbed, so that the decomposition of organic matter in the soil to become humus is very hampered, consequently very few plants get nutrient intake, especially microelement. With the lack of micro elements, the metabolic processes in the body are disrupted, so the plant produces little secondary metabolites that can kill pests and plant diseases (Hoerussalam *et al.*, 2013). Commonly in paddy fields, phosphate is available for low plants, so to provide the availability of phosphate in the soil it is necessary Rhizobacteria from Leguminosae Plants that can dissolve phosphate bound to the soil organic matter granules but also

stimulate the growth of Rhizobium bacteria (Rao, 1994). To increase the growth of *Rhizobium* sp in the soil, it is necessary to find the bacteria that live on the surface of plant roots (Rhizobacteria) of leguminosae and able to improve the growth of Rhiobium bacteria, so that more *Rhizobium* bacteria forming nodule of the root and plants get the nitrogen intake from air by *Rhizobium* sp so that the plant growth becomes fertile and healthy. With the good growth of plants, the plant will produce exudate on the root surface of the plant, the exudate is rich in protein, carbohydrates and vitamins that are needed for survival of Rhizobacteria and *Rhizobium* sp on roots of peanuts

Materials and Methods

Propagation of phosphate solubilizing rhizobacterium

Phosphate Solubilizing Rhizobacterium are derived from several types of leguminous root crops, then bacteria were grown back on the media Pikovskaya + PCNB. Rhizobacteria growing by establishing a clear zone around the colony was phosphate solubilizing rhizobacterium being searched (Jin-Soo Son, *et al.*, 2014; Hefdiyah and Maya Shovitri, 2014). While the efficiency index dissolving phosphate (IEP) by Rhizobacteria can be measured by using the following formula,

$$\text{EIP} = \frac{\text{Diameter of Clear zone}}{\text{Diameter of Colony}}$$

Formulation of biofertilizer with phosphate solubilizing rhizobacterium as active ingredients

Construction of flour formulation as biofertilizer on peanuts plant

The preparation of the formulation was carried out by culturing the Phosphate

Solubilizing Rhizobacterium on liquid PPG medium in a 10-liter fermentor and incubated for 1 week, then microbial colonies harvested by centrifugation with speed of 10000 rpm, then the sediment mixed in a mixture of Milk and bentonite (2: 1) with a concentration of 1% bacteria and dried to dry air using a blower, after dry the mixture in puree back to powdery, then the biofertilizer is ready to use

Preparation of liquid formulation as biofertilizer on peanut plant

Phosphate Solubilizing Rhizobacterium is cultured on Potato Peptone Glucose (PPG) liquid medium and, fermented using biofermentor for 1 week and pH measurement. When the fermentation biopesticide solution showed an acid pH (1.0-5.0), then the solution was added 1 m KOH in order to increase the pH to 7.4. Then the culture mixed with Tween 80 as much as 1% to preserve microbes, then formed liquid biofertilizer of Phosphate Solubilizing Rhizobacterium

Preparation of active sand formulation as biofertilizer on peanut plant

Preparation of active sand formulation was done by preparing active sand media with the procedure of formation is as follows; 500 g Active Sand mixed with 10 grams of cane sugar and water sufficiently. The material is mixed evenly and in the input in a plastic bag to be sterilized with autoclave. After it was inoculated with 10 ml of Phosphate Solubilizing Rhizobacterium was stir well and incubated at room temperature for 2 weeks, to form biofertilizer

Preparation of compost formulation as biofertilizer on peanut plant

Prepared isolates from Phosphate Solubilizing Rhizobacterium respectively in culture on

Potato peptone glucose (PPG) medium and incubated for 2 days until the medium looks cloudy and full of overgrown bacteria, then prepared humus media of raw material derived from cow manure biogas. Humus media packed in plastic bags each as much as 125 g and wood charcoal flour as much as 25 g, then sterilized using autoclave. After cold humus medium, the media was inoculated with 10 ml culture of Phosphate Solubilizing Rhizobacterium. Furthermore, the humus medium that has been inoculated with Rhizobacteria solvent phosphate was incubated for 15 days, while every day the culture was stirred.

Preparation of isolate *Rhizobium* sp as a nitrogen-producing bacteria

Rhizobium isolate bacteria obtained in the study were Rhizobium Btl 8. The bacterium was cultured on a liquid YEM (Yeast Extract Mannitol) and incubated for 2 days. Then the solution of the bacterium is diluted to obtain the concentration of Rhizobium 10^6 cfu / ml bacteria and then 1 ml of Rhizobium bleach inoculated on composite formulation media to be applied to peanut plant together with the various Rhizobacterium formulations of Phosphate Solubilizing Rhizobacterium as above

Application of rhizobacteria as biofertilizer in peanut with seed treatment

Before peanut seeds are planting in the experimental plot, seeds are applying Phosphate Solubilizing Rhizobacterium by way of seed treatment;

a. For the Phosphate Solubilizing Rhizobacterium in formulation form of flour, liquid and active sand as much as 50 g, mixed evenly on 100 g Sterile compost in a humid state, then stirred evenly in a sterile plastic bag and incubated for 24 hours, then in a

sterile plastic bag was inserted each with 50 peanut seeds and 24 hours of Imbibition, then obtained the seeds that have been mixed with biofertilizer.

b. For the Phosphate Solubilizing Rhizobacterium in the form of Compost, peanut seeds of 50 seeds can be mixed with the biofertilizer Compost, and Imbibition for 24 hours, to get seed treatment

c. Application of the bacteria *Rhizobium* Btl 8 may be administered to the seed by mixing peanut seeds which have been treated by Imbibisi Phosphate Solubilizing Rhizobacterium, then the seeds can be directly planted to the experimental plot according to the treatment.

Planting of peanut seeds have been treated seed treatment of Biofertilizer in rice field

Seeds of peanuts that have been treated seed treatment of Phosphate Solubilizing Rhizobacterium planted in the field with plant distance 20 X 20 cm, the soil is processed deeply 15 cm, the size of plot 1 X 2 M, each planting hole filled 3 seeds, and after growing in in reserving become one plant per hole (Fig. 1).

The treatment

a. Biofertilizer Formulation:

1. Flour Formulation (T)
2. Liquid Formulation (C)
3. Active Sand Formulation (P)
4. Compost Formulation (K)

b Type Phosphate Solubilizing Rhizobacterium capable of inducing plant growth:

1. Phosphate Solubilizing Rhizobacterium *Serratia marcescens* (Rb 36)
2. Phosphate Solubilizing Rhizobacterium *Ochrobactrum* sp (Rb 35)

3. Phosphate Solubilizing Rhizobacterium *Achromobacter spanius* (Rb 3)

4. Phosphate Solubilizing Rhizobacterium *Enterobacter cloaceae* (Rb 9)

Plants are well maintained and observations that include

1. High of peanut plant
2. Number of leaves, flowers and Pod
3. leaf chlorophyll content (SPAD units) in peanut
3. Seeds and seed production per Ha
4. Number and weight of root nodules per plant
5. The type and diseases intensity which infect plants,

The intensity of the disease is calculated according to the formula of Boggie and Hans, (1988)

$$I = \sum \frac{(n \times v)}{ZN} \times 100\%$$

Information

I = Intensity of leaf spot disease

n = Number of Plants showing symptoms Leaf spot disease

v = The numeric price value (Score) of each category

Z = Score value of the highest category

N = number of plants diseases

Results and Discussion

The ability of Rhizobacteria from leguminous root to dissolve phosphate

Observations of the ability of Phosphate Solubilizing Rhizobacterium to dissolve the phosphate can be seen in table 2, in get that Phosphate Solubilizing Rhizobacterium Rb 3, Rb9, Rb 35 and Rb36 are the best bacteria in dissolving phosphate and these bacteria are

used for subsequent research

Plant growth as a result of the use of biofertilizer of phosphate solubilizing Rhizobacterium

In Table 3, it was observed that after peanut seeds wrapped as seed treatment with Phosphate Solubilizing Rhizobacterium as biofertilizer in the field, the results obtained that all Biofertilizer treatments were not significantly different to plant height, number of branches and number of chlorophyll produced, but the number of leaves produced by peanut plant showed significant differences between all treatments

In table 3 obtained that the number of leaves on plants treated with Phosphate Solubilizing Rhizobacterium Rb35, Rb36, Rb9 and Rb3, the number of plant leaves is much higher and significantly different from the control.

The biofertilizer formulation in the form of active Sand for Bacteria to produce the largest number of leaves and different from other formulation, possibly sand formulation uses active sand and added sugar ingredients which are microbial food reserves during storage, in addition also looks compost formulation improve life of Phosphate Solubilizing Rhizobacterium to increase plant growth.

Effect of Biofertilizer with the active ingredient of Phosphate Solubilizing Rhizobacterium on vegetative weight of plant

In Table 4 has shown that, usage of Phosphate Solubilizing Rhizobacterium produce weight of the root, weight of dried root and dry weight of vegetative crops, show no significant difference between treatments. But against the number of root nodules produced by *Rhizobium* Sp, after application Phosphate Solubilizing Rhizobacterium, it appears that

all Rhizobacteria formulated in the form of compost, active sand, flour and liquid can increase the number of root nodules and different with control.

This also indicates that the Phosphate Solubilizing Rhizobacterium is possibly also PGPR, capable of producing Auxin Hormone, this hormone will stimulate produce more hair roots, will cause *Rhizobium* Sp easily enter into the roots and form many root nodules as a place of life of *Rhizobium* sp in the event of symbiosis with plants to provide Nitrogen Nutrition for plants.

Phosphate Solubilizing Rhizobacterium Rb35 (*Ochrobactrum* sp), Rb36 (*Serratia marcescens*) and Rb3 (*Achromobacter spanius*) formulated as biofertilizer in the form of active sand has the ability to increase the number of root nodules in peanut plants

The effect of use of biofertilizer with the active ingredient of phosphate solubilizing rhizobacterium to weight of generative phase peanut plant

In Table 5, it appears that the number of pods, weight of pods and Weight Seeds/Plant showed no significant difference between treatment and control.

However, Weight seeds /plot and peanut yield per Ha gave significantly different results between treatments.

However, Rhizobacteria in formulation in the form of active Sand gives the weight of seeds per plot is high compared to other treatments. Similarly, the yield of plants per Ha, the highest can be obtained Rb 36 (*Serratia marcescens*), Rb3 (*Achromobacter spanius*) and Rb 9 (*Enterobacter cloaceae*) in Active Sand formulations.

From the results of this research, it is found

that Phosphate Solubilizing Rhizobacterium Rb 36 (*Serratia marcescens*), which is formulated in the form of Active Sand can increase the yield of peanut plants up to 4.24 Ton per Ha, while other bacterial treatment between 2 to 3 Ton per Ha.

Rb 36 (*Serratia marcescens*) gives the highest yield up to 4.24 Ton per Ha, and it can be concluded Active Sand formulation for Rhizobacteria Rb 36 (*Serratia marcescens*) is the best treatment to increase the production of peanut plant

Effect of biofertilizer with the active ingredient of phosphate solubilizing rhizobacterium to development leaf spot disease on peanut plants

In Table 6, the appearance of leaf blight disease that infects peanut plants, but plants was applied with biofertilizer more resistant to leaf blight disease in comparison control. But it appears also that the rhizobacteria Rb35 (*Ochrobacterium* sp) in the formulation in the form of compost causes plants more resistant to leaf blight disease.

Table.1 Score of Leaf spot disease infection (numerical value) on peanuts (Sarwono, 1995)

Scor	Percentage of disease Svmtoms (%)
0	No symptoms; 0% disease, No symptoms Leaf spots
3	light Symptomatic; 1% - 15% show symptoms Leaf spots
5	Medium Symptomatic; 16% - 35% showing symptoms Leaf spots
7	Weight Svmtoms: 36% - 75% show svmtoms Leaf spots
9	Very Weight symptomatic; 76% - 100% showing symptoms

Table.2 Efficiency Index of Phosphate Solubilizing Rhizobacterium from Leguminosae roots after being cultured in media Pikovskaya + PCNB

No	Phosphate Solubilizing Rhizobacterium from Root Plant of	Phosphate solvents Efficiency Index (IEP)
1	Rb 53 (<i>Cajanus cajan</i>)	4.71
2	Rb 55 (<i>Vigna sinensis</i>)	4.73
3	Rb 38 (<i>Cajanus cajan</i>)	0.11
4	Rb 3 (<i>Stylosanthes guianensis</i>)	8.72
5	Rb 36 (<i>Cajanus cajan</i>)	8.80
6	Rb 35 (<i>Cajanus cajan</i>)	7.75
7	Rb 51 (<i>Cajanus cajan</i>)	4.85
8	Rb 5 (<i>Sesbania grandiflora</i>)	1.44
9	Rb 58 (<i>Vigna sinensis</i>)	0.38
10	Rb 9 (<i>Leucaena glauca</i>)	7.47
11	Rb 6 (<i>Sesbania rostrata</i>)	0.29
12	Rb 8 (<i>Gliricidia sepium</i>)	0.45

Table.3 Effect of various types Biofertilizer Phosphate Solubilizing Rhizobacterium against Plant height, Leaf Amount, Branch number and leaf chlorophyll content on Peanuts

Type of Bacteria and its formulations	High peanut plant (Cm)	Number of leaves*	Number of branches	Chlorophyll content (SPAD)
Rb35K	56.2	60.4a	6.1	38.5
Rb35P	59.3	88.4de	7.8	32.4
Rb35T	56.3	75.3bc	7.9	38.6
Rb35C	66.1	66.1a	7.0	32.2
Rb36K	66.7	90.9e	7.3	35.5
Rb36P	62.0	85.8de	8.3	38.1
Rb36T	55.4	73.3b	7.2	36.7
Rb36C	63.8	90.4e	6.3	30.7
Rb3K	67.9	79.6cd	6.3	36.4
Rb3P	62.1	83.2d	6.6	32.9
Rb3T	59.9	76.6c	7.4	36.8
Rb3C	64.3	75.0bc	6.9	31.7
Rb9K	60.6	67.8ab	6.4	37.0
Rb9P	61.8	83.2d	7.2	34.6
Rb9T	53.8	78.8d	6.6	40.3
Rb9C	56.4	86.3de	6.9	33.3
Control	54.9	73.3b	6.9	34.0

*The same letter in the same column shows no significant difference in DMRT 5%

Table.4 Influence of application different types of Biofertilizer to Number of Roots nodule, Root Weight, Root dry weight and dry weight of vegetative plants

Type of Bacteria and its formulations	Roots nodule/Plant *)	Root Weight (gr)	Root dry weight (gr)	Dry weight of vegetative plants (gr)
Rb35K	73.0c	1.8	0.8	24.13
Rb35P	88.0cd	1.6	0.8	31.85
Rb35T	57.3b	1.6	0.8	32.82
Rb35C	56.7b	1.4	0.8	32.36
Rb36K	72.2bcd	2.6	1.4	27.14
Rb36P	106.1d	2.6	1.2	39.44
Rb36T	88.3d	1.4	0.7	29.30
Rb36C	76.7cd	1.5	0.8	29.23
Rb3K	48.6ab	1.8	0.8	30.37
Rb3P	98.7d	3.8	1.8	38.48
Rb3T	68.1bc	3.5	1.5	28.02
Rb3C	45.2a	2.2	1.1	25.57
Rb9K	79.0cd	1.9	1.0	28.65
Rb9P	43.2a	1.0	0.6	21.79
Rb9T	46.0a	1.6	0.8	36.12
Rb9C	55.9b	2.2	1.2	25.23
Control	40.4a	1.7	0.9	33.26

*The same letter in the same column shows no significant difference in DMRT 5%

Table.5 Effects of various types of biofertilizer on; Number of pods, Weight Pods, Weight Seeds, Dry seed weight and Yield per Ha

Type of Bacteria and its formulations	Number of pods/ Plant	Weight Pods /Plant (gr)	Weight Seeds, /Plant (gr)	Weight seeds /plot (gr)*	Yield Ton/ Ha *
Rb35K	12.3	17.8	17.19	77.46ab	2.53ab
Rb35P	15.4	23.4	24.47	90.25b	2.86b
Rb35T	16.9	17.6	23.28	91.31b	2.57ab
Rb35C	14.1	14.5	24.45	95.45bc	2.68b
Rb36K	14.2	18.0	18.16	93.16b	2.42a
Rb36P	21.4	27.1	31.00	132.21d	4.24e
Rb36T	16.1	19.7	25.17	95.56bc	2.79bc
Rb36C	15.9	17.7	22.16	100.34c	3.25c
Rb3K	13.3	18.9	18.45	106.86c	3.36cd
Rb3P	19.9	25.2	26.90	110.67cd	3.38d
Rb3T	17.0	26.5	20.20	97.61c	3.24c
Rb3C	11.4	16.3	16.23	82.43ab	2.47a
Rb9K	13.0	18.0	22.77	108.47c	3.42de
Rb9P	13.7	18.9	23.28	97.65c	3.16bc
Rb9T	17.4	23.3	21.75	107.58cd	3.27c
Rb9C	12.1	16.4	17.91	72.26a	2.24a
Control	16.4	15.6	18.26	71.12a	2.13a

*The same letter in the same column shows no significant difference in DMRT 5%

Table.6 Effect usage of various types of Biofertilizer against infection of leaf spot disease *Cercospora arachidicola*, *Alternaria arachidis*, leaf Blight *Leptosphaerulina crassiasca* and leaf rust *Puccinia arachidis* on peanut

Type of Bacteria and its formulations	Intensity of leaf disease (%)			
	leaf spot <i>Cercospora arachidicola</i>	leaf spot <i>Alternaria arachidis</i>	leaf Blight <i>Leptosphaerulina crassiasca</i>	Rust Disease (<i>Puccinia arachidis</i>)
Rb35K	16.7	10.6	7.9	27.0
Rb35P	25.8	19.0	18.7	23.2
Rb35T	19.9	14.8	12.0	41.8
Rb35C	21.8	13.9	13.0	34.1
Rb36K	26.7	17.1	16.7	44.8
Rb36P	17.1	13.4	14.4	28.2
Rb36T	19.9	13.9	13.0	25.5
Rb36C	18.5	15.7	13.9	33.0
Rb3K	24.1	18.8	13.4	36.6
Rb3P	20.8	16.7	14.8	25.9
Rb3T	22.2	16.7	15.3	21.3
Rb3C	27.8	20.5	19.6	42.4
Rb9K	23.1	15.7	13.4	25.0
Rb9P	26.9	22.4	16.2	21.0
Rb9T	23.6	14.8	14.4	34.5
Rb9C	23.1	13.0	13.9	32.3
Control	25.0	17.6	17.1	68.75

Figure.1 Plant research in the field



Figure.2 Leaf rust disease on peanuts



Also seen In Table 6, that the application of Phosphate Solubilizing Rhizobacterium for all Biofertilizer formulations can to protect peanut plants from leaf rust disease when compared with control. The best Phosphate Solubilizing Rhizobacterium are Rb35 (*Ochrobactrum* sp), Rb36 (*Serratia marcescens*), Rb3 (*Achromobacter spanius*) and B9 (*Enterobacter cloaceae*) formulated in the form of Active Sand. Thus it can be said that Phosphate Solubilizing Rhizobacterium can induce peanut systemic resistance to pathogen leaf rust disease on peanut plants (Fig. 2).

From the results of this study can be concluded that:

1. Phosphate Solubilizing Rhizobacterium which has been formulated in the form of biofertilizer Flour Formulation (T), Liquid Formulation (C), Active Sand Formulation (P), and Compost Formulation (K), Able to improve plant growth in the form of plant height, number of leaves and number of branches compared to control. Liquid and flour formulations, however, generally promote poor plant growth.
2. The best formulation biofertilizer of Phosphate Solubilizing Rhizobacterium for growth and production of the peanut plants is formulated in the form of Active Sand.
3. However Liquid and flour formulations are generally less good for increasing plant growth and crop yields.
4. The kind of Phosphate Solubilizing Rhizobacterium capable to improve the growth and production of peanut plants are *Serratia marcescens* (Rb 36), *Enterobacter cloaceae* (Rb 9) and *Achromobacter spanius* (Rb 3).
5. Application of Phosphate Solubilizing Rhizobacterium as biofertilizer in peanuts turns out;

- a. Peanut crops that are applied with Biofertilizer Phosphate Solubilizing Rhizobacterium, less able to protect the plant from the infection of leafspot (*Cercospora arachidicola*) leaf spots (*Alternaria arachidis*) and leaf blight (*Leptosphaerulina crassiasca*).
- b. Peanut crops that are applied with Biofertilizer Phosphate Solubilizing Rhizobacterium, able to protect peanut plants from rust disease (*Puccinia arachidis*)

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