



# International Journal of Sciences: Basic and Applied Research (IJSBAR)

ISSN 2307-4531  
(Print & Online)

<http://gssrr.org/index.php?journal=JournalOfBasicAndApplied>



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## Effect of Plant Growth Promoting Rhizobacteria (pgpr) and Liquid Smoke Against Diseases Attacks and Growth of Pepper (*Piper nigrum* L.)

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### Abstract

This assessment is a preliminary study that aims to determine the role of PGPR and liquid smoke to the growth and attack of diseases of pepper plants. The assessment was conducted at Natar, South Lampung, Indonesia, on pepper crops. The study lasts from April to October 2014. The studies used randomized block design consisting of three treatments with nine replications. The treatments ie: A) pepper plants without treatment (control), B) pepper plants that applied with PGPR, C) pepper plants that applied with liquid smoke. The parameters observed were plant height, leaf number, branch number and attacks intensity of curly disease and *Phytophthora capsici*. The observed data for each variable was analyzed by ANOVA and continued with Duncan's Multiple Range Test (DMRT). The results showed that the application of PGPR can be reduce intensity of curly disease up to 61.20% and *P. capsici* intensity can be reduced up to 47.79%. The application of liquid smoke can be reduce the curly disease attack up to 52.28% and *P. capsici* up to 63.87%. Although no significant effect but PGPR and liquid smoke each can increase plant height and leaf number of pepper plants compared with control.

**Keywords:** Pepper; PGPR; liquid smoke; curly disease; *Phytophthora capsici*.

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## 1. Introduction

Pepper (*Piper nigrum* L.) is a plant spices potential and has high economic value in international trade. Few the last years, extensive of planting and production of pepper Indonesia suffered lowering the productivity ranges from 0.6-0.8 kg/plant. One of the factors affecting the decline among other caused the diseases that difficult to overcome by farmers. Some diseases that often attack which can reduce the production of pepper are foot rot disease, yellow disease, and curly disease [16].

Foot rot disease caused by the pathogen *Phytophthora capsici*, the disease can cause plant death within a short time. The typical symptoms of this disease in the form of blue-black at the base of the stem is sometimes accompanied by mucus formation. Symptoms on leaves in the form of jagged black spots like lace on the center or the edges of the leaves. This phenomenon is evident in the fresh leaves and are difficult to observe on the leaves that have dried up or the further symptoms [6].

Yellow disease is caused by the complex circumstances in the form of nematode attack (*Radopholus similis* and *Meloidogyne incognita*), a parasitic fungus (*Fusarium oxysporum*), low soil fertility, as well as soil moisture or water content is low. The disease is mostly found in the Bangka and Kalimantan (Indonesia) and cause yield losses of 80%. Symptoms of yellow disease due to attacks *R. similis* and *M. incognita* visible in the canopy and root damage the soil surface are nodules (gall), the growth of affected plants will be stunted, stiff yellow leaves, and roots damaged. At the higher stage of disease will lead to the leaf stem, fragile, so easily fall and death of vines [1,16,22]. Yellow disease can also caused by viruses such as pepper yellow mottle virus (PYMV) and cucumber mosaic virus (CMV). The disease is not lethal but can hinder growth and cause a drop in production. This disease also characterized by symptoms of young leaves are small and curly pale yellow and mottled [11].

Curly disease is a disease of pepper crucial third after pepper foot rot disease and yellow disease. The cause of the curly disease in Lampung, Indonesia, has been identified as the OLM (organisms-like mycoplasma). Symptoms of the curly disease is new crop attacked early abnormalities on the leaf buds and young shoots, meanwhile, the leaves of which is beneath the normal growing. In plants that have long attacked, shoots leaves coming out of bud showing symptoms of mosaic, shaped leaves into small pieces, wrinkled up curly and generally fragile [9].

If in the root zone of a plant shortage of beneficial microorganisms, it will cause the plants become root to various diseases such as wilt and root rot. In addition the plant will also experience barriers to growth (less fertile). Plant Growth Promoting Rhizobacteria (PGPR) is a bacterium that is able to stimulate root growth and physiology as well as capable of reducing disease or damage by insects [16,17,25]. PGPR can also provide protection against viral diseases. PGPR has many types such as *Acetobacter*, *Azospirillum*, *Bacillus*, *Pseudomonas*, and so on. Application PGPR in agriculture can be used as biocontrol of plant pathogens as well as a biological fertilizer [4]. Reference [10] also reported that the PGPR is a biological agent that has been widely used and tested to control various plant pathogens.

Liquid smoke obtained from condensation by the pyrolysis process of wood constituents such as cellulose, hemi-

cellulose and lignin. The most important group of chemical compounds that produced in the fumigation are phenols, carbonyls, acids, furans, alcohols, esters, lactones and polycyclic aromatic hydrocarbons. Two dominant compounds that act as bacteriostatic is phenol and organic acids are able to control bacterial growth. Phenol is obtained from the pyrolysis of lignin, whereas the organic acids from the pyrolysis of cellulose and hemicellulose. The higher lignin content, the greater the expected phenol obtained. Liquid smoke contains a variety of compounds that can be grouped into groups of phenolic compounds, acids and carbonyl compounds group. Group of these compounds play a role as antimicrobial, antioxidant, giving flavor and color formers. Because of the liquid smoke may play a role as an antimicrobial and antioxidant, then liquid smoke can be used as a preservative, wood anti-fungal and anti-termite and can be used for clotting rubber and natural pesticides [2,24,27,29]. This study was a preliminary study that aims to determine the role of PGPR and liquid smoke to the growth and attack of diseases like *Phytophthora. capsici* attacks and attacks of curly disease of pepper plants.

## 2. Material and Methods

The study was conducted at Natar Experimental Station, South Lampung, Indonesia on pepper crop-old one month after planting. The study lasts from April to October 2014. The studies used randomized block design consisting of three treatments with nine replications so that there are 27 experimental units, each unit consisting of two plants, so overall there are 54 plants. The three treatments were: (A) pepper plants without treatment (control), (B) pepper plants that applied with PGPR, (C) pepper plants that applied with liquid smoke.

PGPR formulation in liquid form derived from the Laboratory of Plant Protection and Horticulture Gadingrejo Lampung, Indonesia, whereas liquid smoke of coconut shell derived from Lampung Assessment Institute for Agricultural Tecknology, Indonesia. Pepper varieties used Natar 2. The method of PGPR application was a dose of 10 ml/liter of water (1.0%), then applied around the roots of pepper once a month, and the method of liquid smoke application was a dose of 5 ml/liter of water (0.5%), dissolved and then sprayed onto the pepper plants until all the leaves and stems wet, sprayed once a month. Fertilizers of NPK applied with a dose of 100 grams per plant per year, and manure 5 kg per plant per year.

Observations were carried out once a month. The parameters observed were plant height, number of leaves, number of branches and the intensity of the *P. capsici* attacks and attacks of curly disease. Plant height observed starting as early planting until the end of the observation, by measuring start stem base to the tip of the leaf. Increased plant height increment is calculated by reducing the plant height at the time of measurement with the plant height at the time of the previous measurement.

Observation of leafs number carried out since the beginning of planting until the end of the observation, by counting the number of leaves there. Increased the number of leaves is calculated by subtracting the amount of leaves at the time of observation by the number of leaves at the time of previous observations.

Observations of the number of primary branch since the beginning of planting until the end of the observation, by counting the number of primary branch there. Increased the number of primary branch is calculated by

subtracting the number of primary branches at the time of observation by the number of primary branches at the time of previous observations.

Observation of the curly disease intensity was done since the beginning of planting until the end of the observation, by counting the number of curl leaf. The intensity of the curly disease was calculated using the formula:

$$I = \frac{a}{a + b} \times 100\%$$

Remarks: I = Attack intensity (%)

a = Number of curl leaf

b = Number of healthy leaves

The intensity of the attacks of *P. capsici* on the leaves was calculated based the formula:

$$I = \frac{\sum_{i=0}^z (n \times v)}{Z \times N} \times 100\%$$

Remarks: I = The intensity of the attack (%)

n = Score

v = The number of leaves with damage

Z = The highest score (5)

N = The number of leaves or leaf sample was observed

The data were analyzed by anova and then Duncan's Multiple Range Test (DMRT).

**Table 1:** Scoring system [14]

Score	Crop Damage Level (%)
0	No attack symptoms
1	> 0 - 20
2	> 20 - 40
3	> 40 - 60
4	> 60 - 80
5	> 80 - 100

### 3. Results and Discussion

#### *The attack of diseases.*

The results showed that the dominant diseases attack the pepper plants even though plants still young were curly disease and *Phytophthora capsici*. In the Natar Experimental Station, South Lampung, Indonesia, the second this disease is endemic in pepper plants. Results of variance showed that application of PGPR, liquid smoke and control this significant at  $\alpha$  1% against intensity of curly disease and significant at  $\alpha$  5% against intensity of *Phytophthora capsici* (Table 2). This indicates that each application of PGPR and liquid smoke have effect against the disease on pepper plants.

PGPR is soil microbes found in the roots of plants which can provide protection against specific pathogens [12]. Chemical compounds contained in the liquid smoke of coconut shell used were predominantly acetic acid (34.77%) and phenol and derivatives (37.25%) (Table 3), which serves to prevent pests and diseases of plants [24].

**Table 2:** Analysis of variance of the intensity of curly disease and *P.capsici* on the treatments of PGPR and liquid smoke.

Source of variation	df	MS		F	
		Curly disease attack	<i>P.capsici</i> attack	Curly disease attack	<i>P.capsici</i> attack
Replication	8	8.852	5.746	0.383	1.579
Treatment	2	245.476	18.227	10.626 **	5.010 *
Error	16	23.100	3.638		
Total	26	35.822	5.409		

\* = significant at  $\alpha$  5% ; \*\* = significant at  $\alpha$  1%

Further analysis showed that the performance of the intensity of the curly disease and *P. capsici* seen higher on the plant untreated (control) (Table 4). This indicates that the application of PGPR and liquid smoke can reduce the intensity of the curly disease and *P. capsici*.

PGPR are bacteria that colonize plant roots, and in doing so, they promote plant growth and reduce disease or insect damage. PGPR have attracted much attention in their role in reducing plant disease. Although the full potential has not been reached yet, the work to date is very promising and many offer organic growers some of their first effective control of serious plant disease [25]. Results of other studies also indicate that PGPR coupled with the provision of Trichoderma and planting *Arachis pintoi* capable of suppressing yellow disease caused by infection with *Meloidogyne incognita* and *Radopholus similis* on a pepper crop is almost 30% higher compare the pepper plants were not given PGPR and *A. pintoi* or pepper plants that use a fungicide [16].

**Table 3:** Chemical compounds contained in the raw material liquid smoke coconut shell

No.	Chemical Compounds	Concentration (%)
1	Acetic acid (CAS) Ethylic acid	33.38
2	Phenol (CAS) Izal	24.10
3	Phenol, 2,6-dimethoxy-(CAS) 2,6-Dimethoxyphenol	1.16
4	2(3H)-Furanon, dihydro-(CAS)Butyrolactone	2.42
5	2-Methoxy-4-methylphenol	3.58
6	Hexadecanoic acid (CAS) Palmitic acid	0.87
7	Ethanol (CAS) Ethyl alcohol	0.14
8	Acetic acid, methyl ester (CAS) Methyl acetate	1.39
9	1,3-Butanediol (CAS) 1,3-Butylene glycol	0.44
10	2,4-Pentadienenitrile (CAS) CYANOBUTADIENE	0.22
11	2-Furancarboxaldehyde (CAS) furfural	3.47
12	1-AXETOXY-CYCLOPENTEN-3-ONE	7.61
13	Methyl Butyric Acid	2.63
14	Undecane (CAS) n-Undecane	1.93
15	Phenol, 4-methoxy-(CAS) Hqmme	11.99
16	Hexane, 1-(hexyloxy)-2-methyl- (CAS) ETHER, HEXYL 2-METHYLHEXYL	2.47
17	2H-Octahydropyrido[1,2-a]pyrazin-1-one	0.35
18	Benzoic acid, 4-hydroxy-methyl ester (CAS) Methyl p-hydroxybenzoate	0.13
19	Butanedioic acid, 3-hydroxy-2,2-dimethyl-diethyl ester (CAS)	0.13
20	2-Heptadecanone (CAS) 2-HEPTADECANON	0.22
21	2-Butanone, 4-cyclohexyl- (CAS) 4-Cyclohexyl-2-butanon	0.16
22	8,11,14-Eicosatrienoic acid, (Z,Z,Z)- (CAS) CIS,CIS,CIS-8,11,14-EICOSATR	0.62
23	TETRACOSANE, 1-BROMO-	0.24

Analyzed in Forest Product Testing Laboratory, Bogor, Indonesia.

PGPR is applied to the roots, giving an advantage in the process of plant physiology and growth. For plants where microorganisms will be very good. The roots are the source of life, there is some exchange of air, nutrients, decomposition and others. In this study, the application of PGPR can reduce the intensity of curly disease until 61.20% compared to the controls, and the intensity of the attacks of *Phytophthora* sp on pepper leaves can be reduced until 47.79% (Table 4).

Research results [13], the application of PGPR can reduce the intensity of PStV attacks of peanuts until 54.69%. Based on these results and the results of previous studies indicate that PGPR can be used as an alternative as the control of plant diseases, but the consistency of effect of PGPR bacteria in the laboratory with on the ground is sometimes different. These bacteria must be propagated and produced in optimum shape during viability nor his biology applied in the field. So some PGPR bacterial inoculation should be re-applied in the field such as Rhizobia.

**Table 4:** Performance of the intensity of the curly disease and *Phytophthora sp.* on the applications of PGPR and liquid smoke

Treatment	The average intensity of the attacks (%)					
	Curly disease	Curly suppression (%)	disease	<i>Phytophthora sp.</i>	<i>Phytophthora sp.</i> suppression (%)	intensity
Control	15.80 a			4.29 a		
PGPR	6.13 b	61.20		2.24 b	47.79	
Liquid smoke	7.54 b	52.28		1.55 b	63.87	

Description: Numbers followed by the same letter in the same column were not significantly different based Duncan Multiple Range Test (DMRT) 5%.

Liquid smoke can serve as a fungicide and bactericide because liquid smoke coconut shell has anti-bacterial compounds, namely phenol and acid. Compound phenol groups, carbonyl and acid groups which simultaneously have antimicrobial properties that can prevent the formation of spores and growth of bacteria and fungi, and inhibits the development of bacteria, fungi and viruses [20,23,24]. Results of research [21], liquid smoke with a concentration of 0.11% can inhibit the growth of the fungus of *Phytophthora sp.* on the cocoa crop by 50%. In this study the liquid smoke with a concentration of 0.5% can suppress the intensity of attack by fungi *P. capsici* on pepper leaves until 63.87% compared with the control, and suppress curly disease until 52.28%.

Liquid smoke coconut shell at a concentration between 0.25-6.0% capable of inhibiting the growth of fungal colonies *Colletotrichum gloeosporoides* and *Fusarium oxysporum*. The results of in vivo tests showed that the liquid smoke with a concentration of 0.5%, 1% and 5%, effectively inhibited the development of anthracnose (*Colletotrichum gloeosporoides*) and fusarium wilt (*Fusarium oxysporum*), up to 100%. However, the liquid smoke use at 5% was not recommended due to inflicting necrosis on cucumber leaves (8). Possible to other plants can also occur necrosis, so we need to watch out for.

### The Growth of Plant

Results of variance showed that application of PGPR, liquid smoke and controls did not differ significantly on plant height, number of leaves and number of branches of pepper (Table 5). This indicates PGPR application and the application of liquid smoke has no effect on plant height, number of leaves and number of branches of the pepper plant. But the results of research [16], stating that the application of PGPR positive effect on the growth of the pepper plant. This can happen because influenced by environmental conditions. In this study, although no significant effect but PGPR and liquid smoke each can increase plant height and leaf number of pepper plants compared with control (Table 6).

Some research, the research result [3] on soybean plants, although it is stated that PGPR no effect on plant height and number of leaves, but it appears that plant height and number of leaves were higher in the treatment

by PGPR compared with those not given PGPR (control ).This is in line with this study, both numerically shown that the treatment of PGPR showed increase plant height, number of leaves were higher than plant that untreated with PGPR (control). Results of research [13], PGPR treatments can improve plant height of peanuts when compared with controls. He also explained the same result is also shown by the research of Mary (2010) that PGPR enhances growth of height and number of leaves of pepper plants.

**Table 5:** Analysis of variance of plant height, number of leaves and number of branches in the PGPR application and liquid smoke application.

Source of variation	df	MS			F		
		Plant height (cm)	Number of leaves	Number of branches	Plant height (cm)	Number of leaves	Number of branches
Replication	8	330.927	128.850	4.104	1.902	0.775	3.135
Treatment	2	382.937	170.954	4.694	2.201 <sup>ns</sup>	1.028 <sup>ns</sup>	3.586 <sup>ns</sup>
Error	16	174.002	166.277	1.309			
Total	26	238.372	155.120	2.429			

ns = not significant

Generally PGPR is helpful in the process of plant physiology and growth. Bacteria in PGPR as biofertilizers can bind nitrogen that can be used by plants so as to increase its growth and also as photostimulator that can directly increase plant growth by producing growth hormones, vitamins and various organic acids as well as improve the nutrition for plants [15,19]. Plant hormones produced by PGPR such as auxin, giberellin and cytokines, as a solvent phosphate and nitrogen fixation, is a compound that makes PGPR also act as biological organic fertilizer [5,26,28].

Liquid smoke has radical quality, which gives the effect of stimulating growth, because the liquid smoke is also an ingredient that functions as a hormone or substances trigger plant growth [18]. But depending on the concentration of the mixture, so that the liquid smoke can also inhibit the growth of plants [7].

**Table 6:** Performance of the growth of plant height, number of leaves and number of branches in the application of PGPR and liquid smoke.

Treatment	The average of accretion		
	Plant height (cm)	Number of leaves	Number of branches
Control	19.81	11.33	1.44
PGPR	32.17	18.06	2.17
Liquid smoke	29.61	19.50	0.72



Description: Numbers in the same column were not significantly different based Duncan Multiple Range Test (DMRT) 5%.

#### 4. Conclusion and Suggestion

##### 4.1 Conclusion

Application of PGPR and liquid smoke can prevent the development of disease intensity curly and *P. capsici* on pepper plants. Application of PGPR at 1% concentration, intensity curly disease can be reduced up to 61.20% and the intensity of *P. capsici* can be reduced up to 47.79% compared to controls. And liquid smoke at 0.5% concentration, intensity curly disease can be reduced up to 52.28% and *P. capsici* up to 63.87% compared to controls. Although no significant effect but PGPR and liquid smoke each can increase plant height and number of leaves of pepper plants compared with controls.

##### 4.2 Suggestion

PGPR can be applied to all phases of plant growth of pepper, start seed treatment, pre-planting soil treatment, until the post-planting phase. Whereas liquid smoke can be applied to post-planting phase of pepper.

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