POTENTIAL EFFECT OF RHIZOBACTERIA *Streptomyces* sp. AND *Bacillus* sp. to GEMINIVIRUS INFECTION AND RED CHILI (*Capsicum annum* L.) QUALITY PRODUCTION

(Pengaruh Potensial dari Rhizobakteria *Streptomyces* sp. Dan *Bacillus* sp. terhadap Infeksi Gemini Virus dan Kualitas Produksi Cabai Merah (*Capsicum annum* L.))

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ABSTRACT

The chili yellow leaf curl disease is one of the biggest obctacle in chili's production in Indonesia. This diseases only transmitted by whitefly (Bemisia tabaci). One of the disease controlling method is by using Plant Growth Promoting Rhizobacteria (PGPR) to reduce pesticide application for the health and environment. The aim of this research is to find out the effectiveness of Streptomyces sp. and Bacillus sp. in supressing yellow leaf curl disease, plant growth and chili harvest. This research has conducted at the experimental farmer's field in Harjobinangun, Pakem, Sleman, Special Region of Yogyakarta. Using varieties of chili Twist 42, Streptomyces isolates which from Bacteriology Laboratory collection's with 10^{-8} CFU/ml and Bacillus isolates which is from Mycrobiology Laboratory collection with 10^{-8} CFU/ml. The result of this research showed that the treatment of combination between Bacillus sp. and Streptomyces sp. could decrease and supress disease incidence and disease intensity if it compared with others treatment. Parameters of plant growth included plant high, stem diameter, root length, root volume and number of productive branch showed that single of Streptomyces sp. and Bacillus sp. without combination were better than combination and untreated. The single application Streptomyces sp. or Bacillus sp. could increase amount of total number and weight of fruits.

Keywords: *Bacillus* sp., *Begomovirus*, chili, PGPR, *Streptomyces* sp.

INTRODUCTION

Chili (*Capsicum annuum* L.) is one of the most important vegetable commodities in Indonesia due to its high economic value. According to BPS (2021) stated that the production of national chili in 2021 is decreasing compare with last year. One of the issues for the chili productivity fluctuation is pest and diseases infection which attacks plants from seedling stage to the harvesting stage of plant.

Yellow leaf curl disease is emerged as a serious constraint to chili production in Indonesia. This disease is caused by Geminivirus (Famili : Geminiviridae) that was reported in Sleman and is known to cause 20- 100% losses to the marketable fruits (Sumardiyono *et al*. 2003). Epidemics of chili yellow leaf curl disease have occurred in several chili growing centers in Central Java, West Java, and the Special Region of Yogyakarta since the early 2000 (Sulandari *et al* . 2006). Geminivirus causes huge yield losses and a decrease in vegetable crop production (Thakur *et al*. 2018). The symptoms of this disases was observed in the field, involving yellow mosaic, mottle, and yellowing. Green mosaic on the top of leaf was the early symptom chili pepper infecting viruses, followed by cupping upward or cupping downward and yellowing, plants will become dwarfs when infected with the virus as the seeds form (Selangga & Distihani 2021).

Therefore, proper countermeasures to control disease caused by Geminivirus are needed. Several efforts to reduce Geminivirus infection are planting varieties that are disease resistant. One way is by inducing resistance by using PGPR (Plant Growth Promoting Rhizobacteria) to enhance plant's resistance.

PGPRs are saprophytic bacterial microorganisms that are free-living in the rhizosphere and colonize the root system aggressively (Ramjegathesh *et al*. 2013). Plant roots with bacteria have a beneficial impact on the development and production of crop plants (Fasusi *et al*. 2021). The beneficial bacterial species is the PGPR that lives in the soil found around the plant's root, influencing plant development and is profitable health-related. They are agricultural biological resources that induce the plant's growth and fruitfulness. They also motivate resistance in plants, a wide range of vegetation of fruits, vegetables, and various forest trees, to different phytopathogens (Zia *et al*. 2020). *Streptomyces* sp. and *Bacillus* sp. are reported as PGPRs.

Bacillus spp. in controlling plant diseases through several mechanisms such as competition, inducing systemic resistance in plants and excreting extracellular metabolites such as antibiotics, cell wall hydrolases and siderophores (Miljakovic *et al*. 2020). Plant root application of *Bacillus subtilis* can triggered ISR against *Tobacco Mosaic Virus* by activating the signaling defense genes (PR-1a and PR-1b), and regulatory genes (NPR1 and Coi1) indicating salicylic acid signaling pathway activation (Wang et al. 2009). According to Arwiyanto *et al.* (2009) also reported the results of his research that the antagonistic mechanism of *Streptomyces* as a biological control agent for bacterial wilt is an antibiosis mechanism by producing antibiotics. *Streptomyces* can inhibit the virus through a variety of mechanisms, including the activation of plant defense pathways and the production of signaling molecules. Those strains induced the systemic acquired resistance (SAR) to virus infections (Majunatha *et al*. 2022). The aim of this research is to find out the effectiveness of *Streptomyces* sp. and *Bacillus* sp. in supressing yellow leaf curl disease, plant growth and chili harvest.

MATERIALS AND METHODS

Study Area

The study was conducted in farmer's field at Cepit, Pakem, Yogyakarta; while other work was done at the Laboratory of Plant Virology, Faculty of Agriculture, Universitas Gadjah Mada. Field study used a Complete Randomized Block Design (RCBD) with three blocks and four treatments, which were *Bacillus* sp., *Streptomyces* sp., combination both *Bacillus* sp. and *Streptomyces,* and untreated (without using biological control and soaked by pure water). Each block was four treatments and 10 chili plants were taken as samples for observation. The variety of chili seeds used in this study was Twist 42. It was planted with spacing 50 cm in row and 60 cm between rows. The chilli seeds were then soaked in a suspension of each treatments. Previously, the seeds were rinsed using pure water to remove commercial coating. They were single treatment of *Bacillus* sp., single treatment of *Streptomyces* sp., combination both of *Streptomyces* sp. and *Bacillus* sp. and untreated by pure water for 60 minutes. 10^8CFU/ml suspension of *Bacillus* sp., *Streptomyces* sp., and their combination were applied into each planting hole before planting.

Diseases Severity and Plant Growth Observation

The observed parameters were root length, root volume, total of productive branches, fruit length, diameter fruit, total of fruit, weight of fruit, total of fruit every harvested, and weight of fruit every harvested. Plant height, stem diameter and diseases severity were observed after fifth weeks during nine times. There were divided into 6 times at the vegetative stages on 6, 7, 8, 9, 10, 11 week after planting and 3 times at the generative stages on 12, 19, 23 week after planting. Yellow leaf curl diasease were observed using the following formula (Ismiyatuningsih *et al*., 2016):

$$DS = \frac{\sum(n \times v)}{N \times Z} \times 100\%$$

DS = Disease severity
n = number of plants or leaves infected
v = disease score
N = number of observed plants or leaves
Z = highest score used

Table 1. Yellow leaf curl diseases was scored using scoring used by Kumar *et al* (2006) with slightly modifications

Score	Description
0	: Healthy Plants (No symptoms)
1	: ≤ 5% curl and yellowing on the upper leaf
2	: > 5% – 25% leaves curl, yellow and begin to swell in the veins
3	: > 25 – 50% leaves curl, yellow and the proportion of malformations leaf (10 – 25%)
4	: > 50 – 75 % leaves curl, yellow and the proportion of malformations leaf (26 – 50%)
5	: >75 % leaves curl, yellow and the proportion of malformations leaf (>50%) and the plants are stunted

Measuring disease incidence can also be using with a formula :

$$Disease\ Incidence = \frac{Number\ of\ plants\ or\ leaves\ infected}{Number\ of\ observed\ plants\ or\ leaves}$$

Data Analysis

The chili crops were harvested at twelves weeks after planting in ten samples. They were harvested and observed every three day times. Chili crops would be divided into five categories; healthy, mild malformation, moderate malformation, severe malformation and rotten. The variables observed were total of fruit and weight of fruit. Proximate test data were analyzed using Analysis of Variance (ANOVA) and Duncan Multiple Range Test (DMRT) with 5% level.

RESULTS AND DISCUSSION

The symptoms were shown for each plant infected with the Begomovirus in the field, but the initial symptoms were shown by yellowing of the leaves or experiencing chlorosis. The severe symptoms of this virus infection are shown by the yellowing leaf curl, cuppings (bowl), leaves malformations and then the plants become stunted. Infection of young plants will make greater yield loss when it was compared to Begomovirus infection at the generative phase (Figure 1).

Figure 1. Symptoms of Begomovirus Infection on Red Chili (*Capsicum annum* L.)

According to Sulandari (2006) the symptoms of yellow leaf curl disease caused vary greatly depending on the cultivar, virus strain and physical environment. Begomovirus infection in chilies has very distinctive symptoms, namely thickening of the leaf veins, leaf edges curling upwards and bright yellow leaf blades. Even though the pathogen does not kill the plant, in severe symptoms it causes new leaves to grow smaller, flowers fall off and do not produce fruit.

Observation of disease development in a single application of *Streptomyces* sp., *Bacillus* sp., a combination of both and controls showed different results. Disease incidence is the ratio of the number of plants infected with the pathogen to the entire plant population. Incidence is indicated by counting the number of unhealthy plants population to the total plants population. While disease severity is the severity

of the disease which is calculated using scoring on plants. The results were not significantly different for both the diseases incidence and severity between all the treatments were showed on Figure 2 and Figure 3.

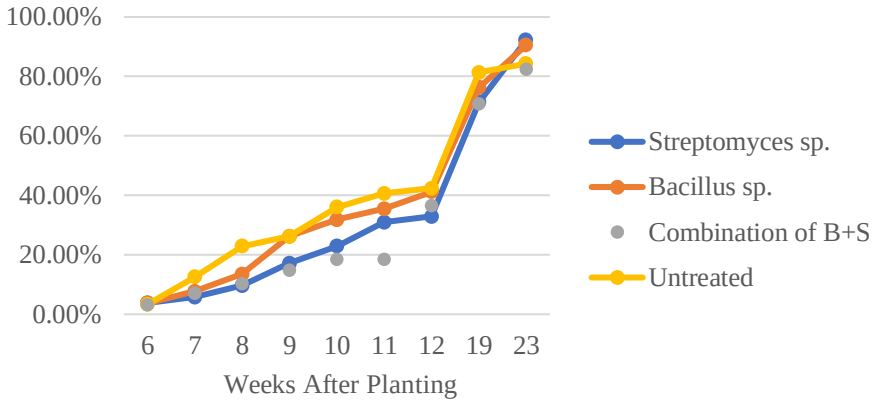

Figure 2. Diseases incidence on red chili (*Capsicum annum* L.)

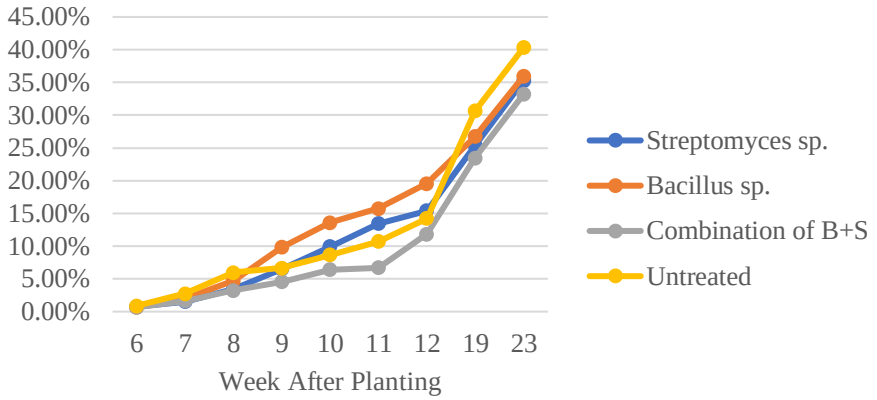

Figure 3. Diseases severity on red chili (*Capsicum annum* L.)

Table 1. Diseases incidence and severity on red Chili

Treatment	Disease Incidence (%)		Disease Severity (%)	
	11st week after planting	23rd week after planting	11st week after planting	23rd week after planting
Streptomyces sp.	30.94 b	92.16 a	13.46 a	35.29 a
Bacillus sp.	35.51 ab	90.54 a	15.75 a	35.96 a
Combination *of Bacillus* sp. and *Streptomyces* sp.	18.44 b	82.38 a	6.68 a	33.22 a
Untreated	52.87 a	84,29 a	13.85 a	40.33 a
CV (%)	0.3444	0.276	0.674	0.674

Description: Test using ANOVA with α = 5%. and tested further DMRT at α = 5%. ns : non significantly

Based on Table 1 the result of diseases incidence at the vegetative stages on 11th week after planting were shown significantly different among the treatments. The combination of *Bacillus* sp. and *Streptomyces* sp. were effective to suppress the development of yellow leaf curl disease in chilli at the initial stage of plantation. But the other results were not significantly different between the treatments both of diseases incidence and severity on 23rd week after planting. Meanwhile, for the diseases severity was showing good trend for yellow leaf curl suppression. According to Widodo *et al*. (2023) states that the

expression of symptoms in plants infected with Begomovirus is also influenced by the virus strain and the time of infection. The chili juveniles can experience decay and be unable to produce when contracting the Begomovirus disease. The treatments used tend to inhibit the diseases severity during the generative stages, so the losses of yield could be decreased. In the experimental garden, *Bemisia tabaci* as vector mostly found at the generative stages Figure 2 and Figure 3.

The growth and development of chili plants were vegetative and the generative stages. The vegetative phase was the phase that starts from seed germination, grows into seedlings and characterized by the formation of the first leaves and continued until the first flowering and or fruiting period. In red chili plants, this stage starts from seed germination until the plants form flower primordia. The generative stages is a phase characterized by shorter growth of twigs and nodes, shorter spacing between leaves on plant shoots, and shoot growth stops (Prihmantoro 2005). There are so many factors that can affect the agronomic parameters in chili plant, especially is pathogens. The diseases caused by Begomovirus has an important roles to inhibit the plant growth in chili. So it was necessary to determine the effectiveness of the application the treatment Table 2.

Table 2. Growth and development on red chili

Treatments	Plant Height (cm)	Stem Diameter (cm)	Number of Productive Branches	Root Volume (ml)	Root Length (cm)
Streptomyces sp.	120.84 [a]	0.99 [a]	144.4 [a]	15.33 [a]	22.03 [a]
Bacillus sp.	99.47 [a]	0.99 [a]	141.6 [a]	22.33 [ab]	28.7 [a]
Combination of *Bacillus* sp. and *Streptomyces* sp.	80.75 [a]	0.91 [a]	138 [a]	8.56 [b]	21.17 [a]
Untreated	80.52 [a]	0.84 [a]	110.8 [a]	6.83 [b]	21.06 [a]
CV (%)	0.881	0.216	0.226	0.0165	0.0667

Description: Test using ANOVA with α = 5%. and tested further DMRTat α = 5%. ns : non significantly.

Parameters of growth and development on red chilli were observed at the end of plantations on 23rd week after planting. The result of root volume was significantly different between the treatments Table 2. The root volume was one of indicator to root growth. *Bacillus* sp. and *Streptomyces* sp. were reported as PGPR that can stimulate plant growth with producing hormones such as IAA. IAA *(Indole-3-Acetic-Acid)* is a growth hormone that plays an important role in plant growth and development. Microbes that are able to produce IAA can increase root growth and extension so that the root surface becomes wider and ultimately plants are able to absorb more nutrients from the soil (Vurukonda *et al.* 2018; Hashem *et al*. 2019).

Based on Figure 4 the appearance of chilli root were shown different size between the treatments. Root morphology of single treatment of *Bacillus* sp. was shown the biggest and the longest root hair than others. IAA hormones can stimulate plants to form lateral roots branching and development of root hairs. These lateral roots aim to expand the area of absorption of nutrients by plants, so that the nutritional needs of plants can also be met (Vacheron *et al*. 2013). According to Khalid *et al.* (2004) stated that wheat rhizosphere bacteria can produce auxin, this hormone can be used by plants to increase root length by up to 17.3 %.

Figure 4. Root morphology of red chili plants (A) Untreated, (B) *Streptomyces* sp., (C) *Bacillus* sp., (D) Combination of *Bacillus* sp. and *Streptomyces* sp.)

The plant height results on Table 2 were not significantly different between the treatments. But they tend to good trend to increase the growth and development on chilli. The other parameters was stem diameter. For the single treatments both of *Bacillus* sp. and *Streptomyces* sp. and their combination were shown that higher score of stem diameter compared to untreated Table 2. The appearance of chilli stem were shown different size between the treatments Figure 4. According to Vurukonda *et al*. (2018) endophytic *Streptomyces* sp. can produce a compound that acts as a plant growth promoter, namely auxin, gibberellins, and cytokinins and many more. Besides that, the bacteria *Bacillus* sp. able to stimulate plant growth because it is known to help plants produce growth hormones such as indoleacetic acid, gibberellic acid, cytokinins, and ethylene in plants (Hashem *et al*. 2019).

The result of number of productive branch was not significantly different between the treatments. According to Gardner *et al.* (1991) states that the number of branches in a plant is influenced by genotype and environment. The availability of nutrients results in better plant growth and easier absorption of nutrients so that plants can form optimal branches so that their growth will also be optimal. As previously explained that the use of antagonistic bacteria *Bacillus* sp. as well as *Streptomyces* sp. and the combination of the two is a bacterium that is able to support plant growth well so that the formation of branches will also occur optimally formed.

Table 3. Fruit length and diameter

Treatments	Fruit Length (cm)	Fruit Diameter (cm)
Streptomyces sp.	10.95 [a]	0.50 [a]
Bacillus sp.	11.25 [a]	0.52 [a]
Combination of *Bacillus* sp. and *Streptomyces* sp.	10.84 [a]	0.52 [a]
Untreated	11.07 [a]	0.50 [a]
CV (%)	0.398	0.895

Description: Test using ANOVA with α = 5%. and tested further DMRT at α = 5%

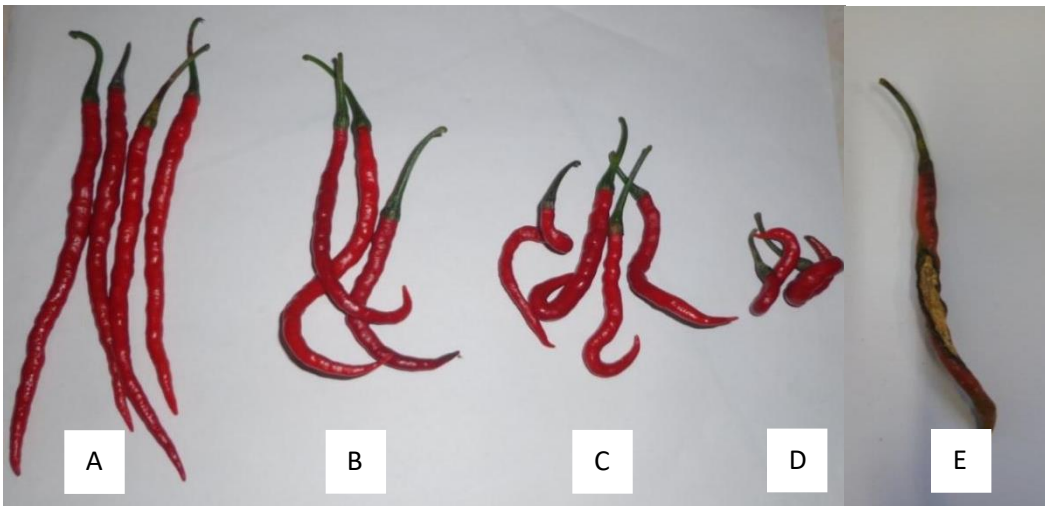

Figure 5. Red chilli fruit criteria (A: healthy, B: mild malformation, C: moderate malformation, D: severe malformation, E: rotten)

PGPR plays an important role in increasing plant growth, yields and soil fertility (Bhat et al, 2023). Based on Table 3. the fruit length and diameter were not significantly different between the treatments. The optimum fruit length of chilli twist varieties is about 15 -17 cm. The results of fruits length showed smaller than the potential length. Measurement of fruit length and diameter were sorted onto several criteria. They were healthy, mild malformation, moderate malformation and severe malformation **Figure 5**. The optimum fruit diameter of twist variety range is 0.8 – 0.9 cm. The highest average yield of chili fruit diameter was shown on the combination treatment of *Bacillus* sp. and *Streptomyces* sp. on the criteria for severe malformation. This severe malformation caused by *Begomovirus* infection which could be assumed changing formation of the chillies and the fruit would become shorter and tend to be larger diameter.

Table 4. The number and weight of chilli fruit yields

Treatments	Number of Chilli Fruit Yields		Weight of Chilli Fruit Yields (gram)	
	Saleable Fruit	Unsaleable Fruit	Saleable Fruit	Unsaleable Fruit
Streptomyces sp.	1328 [a]	270 [a]	2978.7[a]	468.8 [a]
Bacillus sp.	1087 [a]	215 [a]	2734.8 [a]	409.3 [a]
Combination of *Bacillus* sp. and *Streptomyces* sp.	1045 [a]	179 [a]	2161.8 [a]	315.48 [a]
Untreated	1067 [a]	250 [a]	1441.8 [a]	280.56 [a]
CV (%)	0.929	0.786	0.448	0.563

Description: Test using ANOVA with α = 5%. and tested further DMRT at α = 5%.

Saleable fruits were categorized as worth selling to consumer included as healthy, mild malformation and moderate malformation. For unsaleable fruits were included as severe malformation and rotten. The number of saleable chilli fruit yield was not significantly different between the treatments Table 4. Nevertheless, there were positive result using antagonistic bacteria for the treatments especially in single treatment of *Streptomyces* sp. According Ghashash *et al.* (2022) PGPR application can increase plant height, number of leaves, number of branches, number of flowers, number of fruits and fruit weight. *Streptomyces* sp. is also one of the bacteria belonging to the Actinomycetes genus which can produce plant growth hormones which can also act as PGPR. The weight of the fruit is largely determined by the number and size of the fruit, the more the number of fruit and the length of the size, the weight will also heavier (Gashash *et al.* 2022). The heaviest weight of saleable fruits were shown in single treatment of *Streptomyces* sp., and then following by *Bacillus* sp., combination of *Bacillus* sp. and *Streptomyces* sp. and the last one was untreated. This study was proven that single treatment of *Streptomyces* sp. and *Bacillus* sp. was better than combination between *Streptomyces* sp. and *Bacillus* sp. These antagonistic bacteria were play role as PGPR which has a mechanism for producing growth hormones so it can increase

fruit weight. This is in accordance with Putra's research (2014) which states that the population decline that has occurred can be caused by competition for nutrients and living space so that the growth and development of these bacteria is more limited. One of the very large types of Actinomycetes is *Streptomyces* spp. The ability of actinomycetes and *Bacillus* to degrade complex compounds into simple compounds will affect the compatibility of the two bacteria (Putra 2014). It could be assumed that the combination of *Streptomyces* sp. and *Bacillus* sp. was not compatible. Treatment using single of *Streptomyces* sp., *Bacillus* sp. and combinations could suppress the diseases at the initial stages of chilli plant, but at the generative stages could not suppress yet. They could increase the chilli production and growth except the root volume. It could be the bacterial microbes did not survive in the soil for long time. There is also variability in the performance of PGPR that may be due to various environmental factors that might affect their growth and proliferation in the plants. These gaps and limitations can be addressed through use of modern approaches and techniques such as nano-encapsulation and micro-encapsulation along with exploring multidisciplinary research that combines applications in biotechnology, nanotechnology, agro biotechnology, chemical engineering and material science and bringing together different ecological and functional biological approaches to provide new formulations and opportunities with immense potential (Gouda *et al*. 2017).

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CONCLUSION

The treatment of single of *Streptomyces* sp., *Bacillus* sp. and combination both of them could suppress the diseases at the initial stages of chilli plant, but at the generative stages could not suppress yet. It could not be increase the chilli production and growth except the root volume. Single of *Bacillus* sp treatment could increase the root volume in chilli plantation. For another parameters, it tend to good tendency using the treatment compared to untreated.

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