

RESEARCH PAPER

## The use of combination plant growth promoting rhizobacteria to control chili leaf curl disease in the field

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### ABSTRACT

Plant growth-promoting rhizobacteria (PGPR) is a promising technology for controlling viral diseases, including pepper yellow leaf curl disease (PYLCD) of chili pepper caused by *Begomovirus* infection. The objectives of this research were to investigate the effectiveness of PGPR containing *Pseudomonas fluorescens* PF1 and *Bacillus polymyxa* BG25, as well as their combination with other protective agents, to control PYLCD under field conditions in an endemic region. The treatments consisted of a single application of PGPR (a mixture of *P. fluorescens* PF1 and *B. polymyxa* BG25), guano tea, endophytic fungus H5, and neem oil; combination of PGPR with guano tea, endophytic fungus H5, and neem oil; conventional pesticide that relies on synthetic chemical insecticide sprayed weekly; and untreated plots. The experiment was arranged in a randomized block design with four replications. Treatment with PGPR alone was able to delay disease onset by 2.25 weeks, but it caused only a slight reduction in disease incidence. The combination of PGPR + guano tea and PGPR + endophyte H5 provided the best results in controlling PYLCD. The combination of PGPR + guano tea and PGPR + endophyte H5 delayed disease onset by 2.75 weeks and 3.25 weeks, respectively, and reduced disease incidence with effectiveness rates of 52.72% and 52.08%, respectively. These two treatment combinations gave the best performance for plant growth and yield.

**Key words:** *Bacillus polymyxa*, *Begomovirus*, endophytic fungus, protecting agents, *Pseudomonas fluorescens*

### INTRODUCTION

Yellow leaf curl disease, caused by the *Pepper yellow leaf curl virus* (PepYLCV), is one of the most destructive diseases affecting chili peppers in Indonesia. Moreover, this virus is also a global threat to chili pepper production (Czosnek et al., 2017; Thakur et al., 2018). The disease has spread to all chili pepper-producing areas in Indonesia (Selangga et al., 2021; Kesumawati et al., 2019; Tsai et al., 2009). According to the Center for Forecasting Plant Pest Organisms in 2023, the additional area affected by chili disease in 2022 was 14,740.03 ha and is predicted to increase to 20,924 ha in the following year, with yield losses ranging from 30% to 100% (Sulandari et al., 2006).

Various control methods for PYLCD have been applied under field condition, but most

techniques have not provided satisfactory results. Another technology for controlling plant viruses is the use of biocontrol agents through the application of plant growth-promoting rhizobacteria (PGPR). The potential of PGPR for controlling plant viruses, including PepYLCV, has been reported by several researchers. Taufik et al. (2005) worked on the use of *Bacillus subtilis* and *B. stearothermophilus* for *Chili veinal mottle virus* (ChiVMV) and *Cucumber mosaic virus* (CMV) on chili pepper; Damayanti et al. (2007) worked with *Bacillus cereus* I-35 in controlling *Tobamovirus* on chili pepper; Prawiratama et al. (2012) worked on various biocontrol isolates of *Pseudomonas* and *Bacillus* against PepYLCV on chili pepper; and Li et al. (2016) worked with *Enterobacter asburiae* strain BQ9 on TLCV of tomato. The problems with using PGPR are, firstly, the effectiveness in suppressing disease incidence is quite low, below 30% (Prawiratama et al., 2012), and secondly, most studies on the use of PGPR in controlling various plant viruses, including PepYLCV, were conducted in greenhouses or controlled environments.

In addition to PGPR, other environmentally friendly techniques for controlling plant viruses include the use of compost extract (Wahyuni et al., 2010), endophytic fungi (Lestari et al., 2018), and neem oil

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(Vasanthi et al., 2017). Similar to the use of PGPR, these control techniques have shown low effectiveness. It may be necessary to determine whether combining several techniques can increase their effectiveness. Therefore, this research was conducted to investigate the effectiveness of PGPR containing *Pseudomonas fluorescens* PF1 and *B. polymyxa* BG1, as well as their combination with other protective agents, to control PepYLCV under field condition.

## MATERIALS AND METHODS

**Research Site.** Field research was conducted in farmer's field located in Madugondo village, Kajoran district, Magelang regency, Province of Central Java Indonesia. This location is known as the endemic area of PYLCD with high disease incidence (Ariyanti, 2007).

**Seedling Preparation and Plant Maintenance.** TM 999 variety chili seeds were planted in a nursery area covered with a thin cloth to protect the seeds from the initial attack of the whitefly virus. The 24-day-old seedlings were then transplanted into 1 m × 4 m beds covered with black silver plastic mulch, 0.35 mm thick. The spacing between plants was 80 cm × 40 cm. One plot unit consisted of four beds with a total of 80 plants. The crops were irrigated daily by watering. NPK synthetic fertilizer was applied three times during the season: at transplanting, 2 weeks after transplanting (WAT), and 4 weeks after transplanting, with an overall dose of 450 kg/ha. Insect pests were not specifically controlled.

**Treatment and Experimental Design.** The Field experiment was arranged in a randomized block design with nine treatments, each replicated four times. The treatments consisted of a single application of PGPR, guano tea (Primagrain, Wish Indonesia), endophytic fungus isolate H5 (*Cercospora nicotianae*), neem oil (Bali Neem Factory), and combination of PGPR + guano tea, PGPR + endophyte isolate H5, PGPR + neem oil. Comparative treatments included a chemical insecticide (imidacloprid) spray and a control without any treatment. Infection of Begomovirus was confirmed by polymerase chain reaction using universal primers PAL1v 1978 (5'GTATCTGCAGGCCACATYGTCTTYC CNGT3') and PAR1c 715 (5'GATTCTGCAGTD ATRTTYTCRTCCATCCA3') according to Rojas et al. (1993). The PGPR used in the study was a mixture of *Pseudomonas fluorescens* PF1 and *Bacillus polymyxa*

BG25 obtained from Dr. Widodo (Plant Clinic, IPB University). The endophytic fungus isolate H5 is a non-sporulating fungus and was a collected isolate of the author.

PGPR was applied by coating seeds with a bacterial suspension ( $10^6$  cfu/mL) by soaking and then incubating them for 12 hours, followed by drenching the plants (200 cc/plant) two weeks after transplanting. The endophytic fungus H5 was applied by mixing seeds with a mycelial suspension ( $10^5$  cfu/mL), then incubating the seeds in a petri dish containing moistened paper for 12 hours. Guano tea was applied by spraying the plants (1% v/v; 400 L/ha) at weekly interval from 1 to 8 WAT. When PGPR and isolate H5 were combined, treatment on seeds was done by first priming with the endophyte suspension for 12 hours, then coating by PGPR. Conventional treatment plots were sprayed with an insecticide containing the active ingredient imidacloprid weekly.

**Observation.** The population of whiteflies was determined at 2 and 5 weeks after transplanting. Observations were made on the immobile forms of *Bemisia tabaci*, i.e., nymphs and pupae, on two leaves (the third and fourth fully expanded leaves from the top). Three plants were randomly chosen in each plot for sampling the whitefly population. Observations on germination rate, seedling height, and root length were carried out in the nursery on 21-day-old plants. Variables observed on all transplanted plants included disease incidence, onset of diseases, population of whiteflies, plant height, and yield. Disease incidence was measured based on the number of infected plants showing symptoms.

$$DI = \frac{n}{N} \times 100\%$$

DI = Diseases Incidence;

n = Number of plants infected;

N = Total number of plant evaluated.

The onset of disease was determined by the time when symptoms first appeared in each treatment and replication. Plant growth was measured by assessing plant height at 3 and 5 WAT. Ripe fruits were harvested, and the weight per unit was recorded and accumulated for the first six harvests.

**Data Analysis.** All data were analyzed using ANOVA (analysis of variance) followed by DMRT, using the SPSS package program. The effectiveness of the treatments was calculated using the following formula:

$$E = \left( \frac{U - T}{U} \right) \times 100\%$$

E = Effectiveness;

U = Disease incidence in untreated;

T = disease incidence of treatments.

## RESULTS AND DISCUSSION

This study revealed that chili pepper plants with yellow leaf curl from experimental plots were infected by PepYLCV. Amplification of DNA fragments using universal primers for Geminivirus, PAL1v 1978 and PAR1c 715, successfully obtained a DNA band with a size of 1600 bp from a composite sample. Based on the DNA sequence, it was found to have 100% homology with *Pepper yellow leaf curl Indonesia virus* (AB267838.1). The conventional control technique, which relies on weekly sprays, did not show a significant difference from untreated plots in terms of

both disease onset and disease incidence (Table 1 and Table 2). Additionally, neem oil was not effective in controlling leaf curl disease (Table 1).

The mechanism by which PGPR treatments reduce PYLCD in chili peppers was not specifically studied, but it could involve resistance against viruses or resistance against the whitefly vector. The effect on vectors involves suppressing the biological attributes and activity of vector insects (Murphy et al., 2007; Pennel et al., 2005; Hanafi et al., 2007). Murphy et al. (2007) reported that the population of whitefly insects on PGPR-treated tomato plants was less than on untreated plants. This finding is not consistent with this study, in which PGPR and other protecting agents had no significant effect on the amount of whitefly (Table 3). Therefore, the role of PGPR and its combination with other protective agents in controlling the disease is not through the control of whiteflies as vectors. Other research shows that PGPR can be used as a

Table 1. Effect of various protecting agents on incidence of pepper yellow leaf curl disease

Treatments	Disease incidence (%) at week after transplanting									
	1	2	3	4	5	6	7	8	9	10
PGPR	0.00	0.00	0.00 a	0.00 a	0.00 a	0.00	1.90 a	10.13 ab	23.32 ab	28.14 a
Guano tea	0.00	0.00	0.00 a	0.00 a	0.00 a	0.03	1.90 a	11.09 ab	24.45 ab	28.03 a
Endophyte H5	0.00	0.00	0.00 a	0.00 a	0.00 a	0.00	1.78 a	12.73 ab	21.45 ab	27.45 a
Neem oil	0.00	0.00	0.01 a	0.03 a	0.03 a	0.03	3.63 b	14.07 b	28.08 b	32.45 a
PGPR + guano tea	0.00	0.00	0.00 a	0.01 a	0.01 a	0.01	0.61 a	8.25 a	17.45 a	26.03 a
PGPR + endophyte H5	0.00	0.00	0.00 a	0.00 a	0.00 a	0.00	0.45 a	9.08 a	18.24 a	23.01 a
PGPR + neem oil	0.00	0.00	0.00 a	0.01 a	0.01 a	0.01	2.45 b	15.41 b	24.32 ab	33.08 a
Conventional	0.00	0.00	0.01 a	1.99 a	2.01 a	2.01	3.30 b	14.09 b	28.73 b	34.54 a
Untreated	0.00	0.00	0.03 a	2.01 a	2.01 a	2.45	2.45 b	14.41 b	29.24 b	34.45 a

Value in the same column followed by same symbol was not significantly different with DMRT with  $P > 0.05$ .

Table 2. Onset of pepper yellow leaf curl disease with various combination of protecting agents treatments

Treatments	Onset of disease (week after transplanting)	
	Range	Mean
PGPR	7–8	7.50 b
Guano tea	5–8	7.00 b
Endophyte H5	7–9	8.00 b
Neem oil	3–8	6.25 ab
PGPR + guano tea	6–9	7.50 b
PGPR + endophyte H5	7–9	8.00 b
PGPR + neem oil	4–8	6.75 ab
Conventional	3–7	4.75 a
Untreated	3–8	4.75 a

Value in the same column followed by same symbol was not significantly different with DMRT with  $P > 0.05$ .

Table 3. Populations density of white fly (*Bemisia tabaci*) with various combination of protecting agents treatments

Treatments	Amount per leaf at 2 WAT		Amount per leaf at 5 WAT	
	Nymph	Pupae	Nymph	Pupae
PGPR	1.25 a	0.08 a	2.69 a	1.80 a
Guano tea	1.25 a	0.08 a	2.38 a	1.46 a
Endophyte H5	1.17 a	0.00 a	2.47 a	1.69 a
Neem oil	1.33 a	0.17 a	2.15 a	1.46 a
PGPR + guano tea	1.17 a	0.17 a	2.85 a	1.86 a
PGPR + endophyte H5	1.42 a	0.33 a	3.08 a	2.00 a
PGPR + neem oil	1.33 a	0.00 a	2.69 a	2.03 a
Conventional	1.25 a	0.25 a	3.00 a	1.94 a
Untreated	1.42 a	0.25 a	3.08 a	2.15 a

Value in the same column followed by same symbol was not significantly different with DMRT with  $P > 0.05$ . WAT = weeks after transplanting.

resistance inducer against virus infection (Sofy et al., 2019; Soesanto et al., 2014; Beris et al., 2018; Meena et al., 2020). PGPR mediates plant resistance (Hahm et al., 2012) against virus infection by increasing the defense enzyme peroxidase (PO) and polyphenol oxidase (PPO) (Karthikeyan et al., 2024; Abdelkhalek et al., 2022).

Individual treatments show that under field condition and natural infestation, PGPR consisting of *P. fluorescens* PF1 and *B. polymyxa* BG25 was able to control yellow leaf curl disease caused by PepYLCV by delaying the onset of the disease by approximately 2.5 weeks (Table 2) and significantly suppressing disease incidence significantly at 7 WAT (Table 1). It can be said that the level of effectiveness of PGPR is still low, with an effectiveness rate of 22.45%. Endophytic fungi and PGPR may act through similar mechanisms in controlling plant viruses by vector insects (Vidal, 1996; Pennel et al., 2005) and inducing plant resistance. Endophyte H5 was previously reported to have the ability to prolong the incubation periods of PYLCD in chili peppers and reduce severity under controlled conditions (Lestari et al., 2018). For comparison, the endophytic *Fusarium* mediates banana resistance against fusarial wilt by increasing the defense enzymes peroxidase, polyphenol oxidase, phenylalanine ammonia-lyase (Damodaran et al., 2023).

Other individual treatments that significantly controlled yellow leaf curl disease were guano tea and endophyte H5. Guano tea delayed disease onset by 2.25 weeks longer than the untreated control. In addition, endophyte H5 treatment delayed disease onset by 3.25 weeks. Both treatments significantly reduced disease incidence at 7 WAT (Table 1). When PGPR was combined with guano tea or H5, disease

control performance was considerably higher, delaying disease onset by 2.75 weeks and 3.25 weeks respectively. The two combinations had high disease control ability, significantly reducing disease incidence at three observation dates: 7, 8, and 9 WAT (Table 1). Treatments combining PGPR with guano tea and PGPR with endophyte H5 had average effectiveness rates of 52.72% and 52.08%, respectively.

The mechanism by which guano tea controls plant viruses is largely unknown. Guano tea contains a complex mixture of macro and micronutrients, as well as various organic compounds. It has a significant content of Zn, Mn and B, which may play a role in plant resistance against viruses infection (Pennazio & Rogero, 1997; Scheuerell & Mahaffee, 2002). The application of nutrients such as Mn, Cu, and B is known to reduce disease severity by inducing the resistance within the plant, a process called systemic acquired resistance (SAR). This occurs by releasing  $Ca^{2+}$  cations from cell walls, which interact with salicylic acid and activate the plant's defense mechanism (Gupta et al., 2017). Zn increases the signaling of various defense pathways, such as the salicylic acid-dependent pathway and the jasmonic acid/ethylene-dependent pathway. It also improves membrane integrity, which helps in defense against pathogen attacks. Zn deficiency makes plants susceptible to infections, while excess Zn negatively impacts growth and defense due to toxicity (Bastakoti, 2023). Similar substances to guano tea, like compost tea, with various preparation techniques, can mediate tomato resistance against *Cucumber mosaic virus* (Wahyuni et al., 2010; Wajdi et al., 2018).

All treatments had no significant effect on the number of whiteflies per leaf. Table 4 show that, in all observations at 2 and 5 weeks after transplanting, the

numbers of nymphs and pupae were not significantly different. Besides affecting disease, the application of PGPR and endophyte H5 provide better seedling performance and plant growth in the field (Table 4). The

Table 4. Effect of various protecting agents on seeds germination rate and seedlings growth of chili pepper

Treatment	Germination rate (%)	Seedlings height (cm)	Seedlings root length (cm)
PGPR	92.5 b	5.03 b	4.70 b
Guano tea	84.38 a	4.50 ab	3.73 a
Endophyte H5	93.13 b	5.10 b	4.58 b
Neem oil	84.38 a	3.93 a	3.63 a
PGPR + guano tea	91.25 b	4.95 ab	4.68 b
PGPR + endophyte H5	98.75 b	5.68 b	4.43 b
PGPR + neem oil	90.00 b	5.18 b	4.48 b
Conventional	83.13 a	3.98 a	3.78 a
Untreated	82.50 a	4.00 a	3.90 a

Assessment was conducted at 21 days after sowing. Value in the same column followed by same symbol was not significantly different with DMRT with  $P > 0.05$ .

Table 5. Effect of various protecting agents on plant height of chili pepper

Treatments	Plant height (cm) at age of	
	3 WAT	5 WAT
PGPR	35.40 b	102.42 c
Guano tea	28.85 a	85.42 ab
Endophyte H5	30.35 ab	95.70 b
Neem Oil	25.20 a	80.34 a
PGPR + guano tea	35.63 b	99.72 c
PGPR + endophyte H5	31.72 ab	101.71 c
PGPR + neem oil	35.20 b	98.22 b
Conventional	24.85 a	84.52 ab
Untreated	25.20 a	86.42

Value in the same column followed by same symbol was not significantly different with DMRT with  $P > 0.05$ .

Table 6. Effect of various protecting agents on fruit production of chili pepper

Treatment	Harvested fruits per plots (kg)
PGPR	21.35 b
Guano tea	14.99 a
Endophyte H5	21.75 b
Neem oil	8.46 a
PGPR + guano tea	17.23 a
PGPR + endophyte H5	22.27 b
PGPR + neem oil	17.86 b
Conventional	9.42 a
Untreated	9.67 a

Weight of fruits were measured from six harvesting time. Value in the same column followed by same symbol was not significantly different with DMRT with  $P > 0.05$ .

germination rate of chili pepper seeds increased with the treatment of PGPR (Syamsuddin et al., 2022) and endophyte H5 (Lestari et al., 2018). In general, PGPR, endophytic fungus H5, PGPR + guano tea, PGPR + H5, and PGPR + neem oil treatments promoted chili pepper growth, as indicated by an increase in plant height (Table 5). Other treatments had no significant effect on chili pepper growth. The final treatment with individual PGPR, endophyte H5 and the combination of PGPR + H5 and PGPR + neem oil resulted in much higher yields compared to other agents, as indicated by the higher weight of fruit harvested (Table 6). The increasing seedlings performance, plant growth, and decreased yellow leaf curl disease incidence provided by the combination PGPR + guano tea and PGPR + endophyte H5 resulted in significantly better yields of treated chili peppers.

PGPR, consisting of local isolates *P. fluorescens* PF1 and *B. polymixa* BG25, was able to control PYLCD under field condition. PGPR alone can suppress the disease with an efficacy rate of 22.05% based on incidence and delayed onset of disease by 2.5 weeks. While the delay in disease onset by PGPR is considerable, its effectiveness rate of 22.05% is still low for practical proposes. However, when PGPR is combined with guano tea and endophyte H5, the combination shows a relatively high control effectiveness rate, indicated by delaying disease onset 2.75 weeks and 3.25 weeks, respectively. In addition, the combination of PGPR with guano tea and PGPR with endophyte H5, significantly reduces disease incidence, with high effectiveness rates as high as 52.72% and 52.08%, respectively. The high effectiveness of the combination is attributed to the cumulative individual effects of PGPR, guano tea, and endophyte H5. To our knowledge, this is the first report of the combination of PGPR + guano tea and PGPR + endophyte H5 such high effectiveness.

## CONCLUSION

PGPR treatment alone was able to delay disease onset but only caused a slight decrease in disease incidence. The combination treatment of PGPR + guano tea and PGPR + endophytic H5 gave the best results in controlling PYLCD. The combination of PGPR + guano tea and PGPR + endophytic H5 delayed the onset of disease by 2.75 weeks and 3.25 weeks, respectively, and reduced the incidence of disease with effectiveness levels of 52.72% and 52.08%, respectively. Both treatment combinations provided the best performance for plant growth and yield.

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## AUTHORS' CONTRIBUTIONS

SYW, SHH and SBR conceived and designed the analysis, SYW and SBR collected the data. SHH performed the analysis. SBR contributed data or analysis tools. SYW, SHH, and ASN wrote the paper.

## COMPETING INTEREST

I/We have no known competing financial interests or personal relationships that could have influenced the work reported in this paper.

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