

Effect of *Pseudomonas spp* on infection of *Peronosporaparasitica* (Pers. Fr), the pathogen of downy mildew on Chinese cabbage

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Abstract. This research was conducted to study the effect of the application of *Pseudomonas spp* on infection of *Peronosporaparasitica* (Pers.Fr), the pathogen of Downy mildew on Chinese cabbage. The research was conducted in the laboratory and greenhouse Department of Plant Pests and Diseases Faculty of Agriculture Sriwijaya University, Indralaya, Oganllir South Sumatra Indonesia. The research was conducted in the laboratory and greenhouse Department of Plant Pests and Diseases Faculty of Agriculture Sriwijaya University, Indralaya, Oganllir South Sumatra Indonesia. The research was conducted using Completely Randomized Design with ten treatments including control.ie: isolate A, Isolate B, isolate C, isolate D, isolate E, isolate F, isolate G, isolate H, isolate I and control.Each treatment consists of four replications. Results of the study showed that the application of *Pseudomonas spp*. can suppress the infection of *P. parasitica* on Chinese cabbage. The lowest disease intensity was shown by treatment C (isolate *Pseudomonas sp.*)which was significantly different from control.The best treatment in suppressing disease severity of downy mildew on chinese cabbage was isolate H which had disease severity of 37.07 percent, which was significantly different from control and other treatment.

Keywords : *Pseudomonas sp*, infection, *Peronosporaparasitica*, Chinese cabbage.

1. Introduction

Chinese cabbage (*Brassica juncea* L.) better known as caisin in Indonesia is a vegetable crop that is widely known to the public. This plant includes the most popular vegetables because it tastes good and contains lots of vitamins and minerals [1,2]. In the cultivation of these vegetables there are often constraints that limit production, one of which is the attack of downy mildew disease. Downy mildew caused by *Peronosporaparasaitica* (FersFr) can attack planted cabbage in any stage. Losses that can be caused by the attack of this disease can be 24 to 48% [2,3].The disease can destroy the quality of the leaves, also the quantity of the harvest isreduced due to the downy mildew infection. Young seedlings can die as a result of the infection.

Downy mildew is an obstacle in the cultivation of vegetables such as Chinese cabbage. This disease that can be found throughout a horticultural cultivation area is a member of the genus *Brassica*. In the development of the cotyledon, which may turn yellow, shrivel and die [3]. Downy mildew of vegetable brassica crops is widespread and can cause extensive crop damage. The first symptoms of



foliar infection are dark specks that are often irregular in shape, may appear net-like, and are usually accompanied by leaf yellowing. These yellow areas enlarge and, on becoming limited by the leaf veins, take on an angular shape. If the disease is severe the yellow lesions coalesce, resulting in much of the leaf becoming brown[4]. Control of disease is generally done by spraying fungicides, but the facts show that the use of chemicals continuously will result in negative impacts on the environment, increase production costs and leave residues that are harmful to the health of. According to [5], Chemical control of disease disturbs environment, subverts ecology, degrades soil productivity, mismanage water resources. The use of chemicals for fertilizer and crop protection can disturb soil equilibrium, impoverishment of soil microbial communities decreases enzyme activity, impairs nutrient absorption by root, decreases plant resistance to pathogens and lowers soil quality [6,7].

The use of microbial antagonists has been reported to have the potential to be developed as a bio-pesticide to control plant pests and diseases. Bio-control provides an alternative means of reducing these pathogens, which are otherwise difficult to control due to their survival strategies. The renewed interest in bio-control is due to its environmental friendliness, along lasting effect and safety features. Biological control is controlled by reducing the population of pathogen activity or inoculum, both active and dormant by using one or more types of microorganisms, both external and environmental manipulated, host and antagonist. Biological control by antagonistic bacteria can occur through one or more of the following mechanism: competition, antibiosis, hyper-parasite, induction of plant resistance and spurring plant growth. Both external and environmental manipulated, host and antagonist. The mechanism of antibiosis is an inhibition of pathogens by metabolic substances produced by antagonist agents such as: enzymes, volatile compounds. Competition mechanism is a process of suppressing pathogen activity by antagonists against limited sources such as organic matter, inorganic substances, space and other growth factors. The mechanism of hyper-parasite is the destruction of pathogens by substances produced antagonists such as chitinase, cellulase, glucanase and other lethal agents [8,9].

Biological control through the exploration and identification of antagonistic agents to find an alternative to controlling plant pests and diseases has long been realized but not yet adopted widely. Many types of microbial origin or rhizosphere soil either bacteria or fungi are known to be used as a biological control agents such as *Bacillus spp*, *Penicillium spp*, *Trichoderma spp*, *Gliocladium*, *Verticillium*. *Pseudomonas spp* has been shown to inhibit the development of several plant pathogens. *Pseudomonas sp.* are able to inhibit development of *Uromyces salmoneus* the pathogen in rubber plant up to 67% [10]. Bacteria that enhance plant growth and yield are referred to as plant growth promoting bacteria (PGPB) or plant growth promoting rhizobacteria (PGPR). They can produce phytohormones, improve field emergence, increase seed vigor and availability of soil nutrients, enhance plant resistance to abiotic stress and induce defense mechanisms against the plant pathogens. The beneficial microorganisms can establish symbiotic relationships with the root system by colonizing the rhizosphere or the rhizoplane. PGPB exert positive effect on plant health and crop yield and can be used in biological control [11,12,13,14]

According to [15], *Pseudomonas fluorescens*, *P. aeruginosa*, *P. maltophilia* and *Klebsiella pneumoniae* are able to protect plants against Fusarium disease and promote plant growth on agarwood plant. *Pseudomonas spp* are widely used as a biological agents to control airborne contagious pathogens. These bacteria can produce antibiotic compounds such as chitinase enzymes that can hydrolyze cell walls of pathogenic fungi, siderophores and other antibiotics [16,17]. *Pseudomonas fluorescens* widely used in spurring growth, resistance induction and control of plant diseases because they have antagonistic properties and can suppress disease progression by competition of Fe (III) elements and carbon elements, producing HCN, stimulating accumulation phytoalexin so that plants are more resistant and colonize the roots and stimulate the growth of plants. This research was

conducted to study the effect of the application of *Pseudomonas* spp on infection of *Peronosporaparasitica* (Pers.Fr), the pathogen of Downy mildew on Chinese cabbage.

2. Material and methods

The research was conducted in the laboratory and greenhouse Department of Plant Pests and Diseases Faculty of Agriculture Sriwijaya University, Inderalaya, OganIlir South Sumatra Indonesia. The study was conducted using Completely Randomized Design with 9 treatment of *Pseudomonas* spp isolate (A, B, C, D, E, F, G, H and I) and control with 4 replications.

2.1 Isolation *Pseudomonas* spp

Isolation of antagonistic agents is done by taking the soil in the healthy caisinrhizosphere plant located in Palembang. Isolation is done by serial dilution method. Results of the isolation were a number of isolates that were given codes based on the locality names of their origin. The soil weighed as much as 10 g and then put into the Erlenmeyer tube which has contained 90 ml of sterile water. This mixture is homogenized by using a shaker at a rate of 150 rpm for 30 minutes. Suspension diluted up to 10^{-7} . Furthermore, as much as 0.1 ml of the suspension of 10^{-5} - 10^{-7} dilutions were each incorporated petridish which already contained a sterile king'B medium, flattened and then incubated for 3-4 days. The bacterial colonies that grew were subsequently purified, the characteristics of which were examined were physiological test and then ready to be used in the research.

2.2 Preparation of host plant

Chinese cabbages seeds used are local chinese cabbage. Before planting the seed sterilized surface by soaking the seeds in 1% NaOCl for 1 minute, then the seed is washed with sterile water and dried wind. Further seeds are sown on a sterile planting medium consisting of a mixture of sand, soil and manure (1: 1: 1). After one week of age, the seeds were treated with the application of isolate antagonism (A, B, C, D, E, F, G, H and I) by root immersion in antagonistic isolate suspension with spore density 10^7 /ml for 4 hours. Furthermore the seedlings were transferred into polybag that has been filled with sterile media. Inoculation of *P. parasitica* was performed a week after application of *Pseudomonas* spp. isolate by spraying the suspension of *P. parasitica* with spores density 10^4 /ml to the chinese cabbage root evently.

The parameters observed in this research were disease intensity, disease severity and biomass weight of each plant. Disease intensity was calculated using leaf infected percentage, while disease severity using a formula by [18]. Biomass weight was measured using scales. The data were analyzed using the analysis of variance (ANOVA), with the Duncan's Multiple Range Test (DMRT) comparison among means [19].

3. Result and discussion

3.1 Result

3.1.1 Disease intensity. The analysis result of the effect of *Pseudomonas* spp. application on the disease intensity showed a significant effect. Application of *Pseudomonas* sp. Isolate A can reduce the intensity of downy mildew disease up to 59.49 percent, which is significantly different with the treatment of C, E, G, I and control. Between the treatments of H, B, F, D and A were not significantly different from each other, also between treatment C, E, G and I (Table 1).

Table 1. Effects of *Pseudomonas* spp on disease intensity of downy mildew on Chinese cabbage.

Treatment	Disease intensity (%)
Control	76.35 ^a
Isolate C	74.00 ^{ab}
Isolate E	72.72 ^b
Isolate G	73.00 ^b
Isolate I	70.46 ^b
Isolate H	69.23 ^{bc}
Isolate B	63.21 ^c
Isolate F	60.40 ^c
Isolate D	60.13 ^c
Isolate A	59.49 ^c

^{a,b,c} The figures followed by the same letter in the same column mean there is no significant different at $p \leq 0.05$ DMRT.

3.1.2 Disease severity. The application of bacteria *Pseudomonas* spp as biological control of pathogen downy mildew on Chinese cabbage showed a significant effect on disease severity. All treatments of bacterial application of *P. Pseudomonas* spp showed an influence on disease severity suppression compared with control. Isolate H showed the highest suppression of *P. parasitica* infection by 37.07 percent followed by isolate C, E, G and A which was significantly different with control with 43.41%, 47.83%, 47.90% and 48.77% (Table 2).

Table 2. Effects of *Pseudomonas* spp on disease severity of downy mildew on Chinese cabbage

Treatment	Disease severity	Relative percentage to the control (%)
Control	71.36	-
Isolate D	55.51	22.21
Isolate I	53.52	25.00
Isolate F	51.62	27.80
Isolate B	51.46	27.88
Isolate A	48.77	31.65
Isolate G	47.90	32.87
Isolate E	47.83	32.97
Isolate C	43.41	39.16
Isolate H	37.07	48.05

^{a,b,c,d,e,f,g,h} The figures followed by the same letter in the same column mean there is no significant different at $p \leq 0.05$ DMRT.

3.1.3 Plant Biomass. Application of *Pseudomonas* spp as biological control agents tended to increase the weight of plants biomass compared to the control. The highest biomass weight was showed by the Chinese cabbage given F isolate treatment 1 gram/plant followed by D, G, H and I treatment with a mean biomass weight of 0.83 gram/ plant (Figure 1).

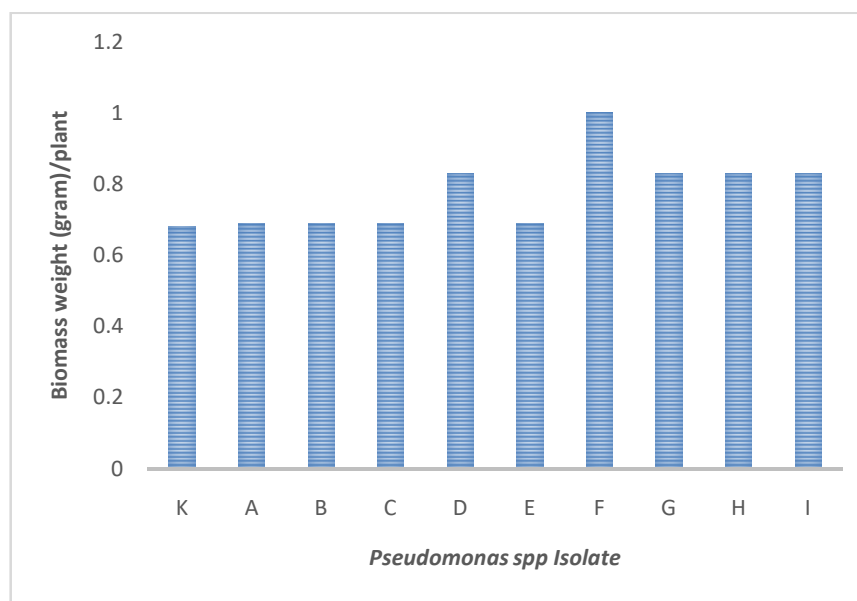


Figure 1. Effect of *Pseudomonas* spp on Chinese cabbage biomass weight.

3.2 Discussion

The suppression of the intensity of downy mildew attack may be related to the ability of bacteria to produce secondary metabolic thus protecting plants from pathogen attack[20]. According to[21], that most antagonistic bacteria have ability to adapt to their environment. The successful of biological control of plants diseases is due to some inhibitory feeding which is commonly found in biological agents such as siderophore, antibiotics, competition, enzyme, toxin and as plant growth promoting rhizo-bacteria (PGPR).[22]reported that beneficial rhizo-bacteria are known to colonize rapidly and aggressively the root system, suppress pathogenic microorganism, and enhance plant growth and development. Under certain condition antibiotics improve the ecological fitness of these rhizo-bacteria in rhizo-sphere, which can further influence long-term biological control efficacy[23]. Some of *Pseudomonas* spp are capable to inhibiting the growth of soil borne pathogens. The inhibition mechanisms for the inhibition of pathogen proliferation are ability to colonize the root, production of antibiotics and enzymes that degrade pathogen cell wall, production of siderophores and hydrogen cyanide [24,11,25].

Natural bioactive compounds produced by antagonistic bacteria can be beneficial to plant health or agricultures. Species of bacteria can resist the plants pathogen in various ways such as by producing antibiotic compounds[26,27]. It has been reported that *Pseudomonas* spp are known to be agents capable of inducing plant resistance, as antagonists and as plant growth promoting[8,28]. According to[28,30] genus *Pseudomonas* can possess a high potential for fixing nitrogen, solubilizing phosphate, inhibiting the development of phytopathogen, producing phytohormones and colonizing root systems. The suppression of the pathogen by antagonistic bacteria occurred because the bacteria are able to remove antibiotics such as pyoverdine, pyoluteorin, 2,4-diacetylphloroglucinol and monoacetylphloroglucinol that could inhibit the growth of pathogen. Some of *Pseudomonas* spp could also inhibit the development of the disease by colonized roots, nutrient competition of iron (III) and carbon element, HCN production, IAA, stimulate phytoalexin accumulation so that the plant became resistant and stimulated plant growth[30,31,32]. This is in line with results of this study where the applied of *Pseudomonas* spp isolate are able to suppress the intensity and severity of downy mildew disease on Chinese cabbage. Increased wet weight is also occurring but not so significant.

4. Conclusion

From the study, it can be concluded that the application of *Pseudomonas spp.* can suppress the infection of *P. parasitica* on chinese cabbage. The lowest disease intensity was shown by treatment A(isolate *Pseudomonas sp.*) which was significantly different from control. The best treatment in suppressing disease severity of downy mildew on chinese cabbage was isolate H which had disease severity of 37.07 percent, which was significantly different from control and other treatment.

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