THE EFFECT OF FLUORESCENT PSEUDOMONADS APPLICATION ON THE RESISTANCE OF CHILI PLANTS AGAINST THE ATTACK OF *RALSTONIA SOLANACEARUM* AND *FUSARIUM OXYSPORUM* IN THE FIELD

Yenny Wuryandari, Sri Wiyatiningsih, and Agus Sulistyono
University of Pembangunan Nasional "Veteran" Jawa Timur
Surabaya, East Java, Indonesia
E-Mail: yennyupn@yahoo.com

ABSTRACT

Chili (*Capsicum annuum L.*) is one of the vegetable commodities with high economic value. Until now, the necessity rate of chili is still high but the production is still low. One of the obstacles, which highly influence chili production, is wilt disease due to *Ralstonia solanacearum* and *Fusarium oxysporum*. This study aims at identifying the best fluorescent pseudomonads isolate in suppressing the development of the primary wilt disease due to *Ralstonia solanacearum* and *F. oxysprorum* in the field. This study employs Completely Randomized Design that comprises 3 treatments. The three treatments are the type of fluorescent pseudomonads which is Pf-122 isolate, Pf-160 isolate, and Pf-B isolate. The observation parameter on suppressing the disease development is perceived from incubation period of the initial appearance of symptoms, disease index and the change of stem tissue color. The result of the study shows that the longest incubation period is treatment with fluorescent pseudomonads of Pf-122 isolate and Pf-160 isolate. The chili is treated with fluorescent pseudomonads of Pf-122 isolate and Pf-160 isolate, the initial appearance of symptoms at the 8th day after inoculation. In the treatment with fluorescent pseudomonads of Pf-122 isolate and Pf-160 isolate compared to control, is able to delay the appearance of symptom for 3 days. The disease development starts from 10th to 30th day, the treatment with Pf-122 isolate shows the slowest development compared to other treatment and control. In the last observation, the disease index with treatment of Pf-122 isolate is the lowest which is 37%, then it is followed by Pf-B and Pf-160 which are 39% and 40% respectively, whereas in the control it reaches 67%. For the color change on stem tissue, there is no real difference of the three treatments of fluorescent pseudomonads application but it differs and smaller from the control.

Keywords: fluorescent pseudomonads, *Ralstonia, Fusarium*

INTRODUCTION

Chili productivity in Indonesia is currently low. The factors causing low production are the use of less qualified seeds, non-efficient cultivation technique and the chili cultivar cultivation which is not resistant to pests and diseases (Soelaiman & Ernawati, 2013). The primary barrier which highly influences the chili production is the main disease, wilt disease *Ralstonia solanacearum* and *Fusarium oxysporum*. Disadvantages from wilt disease of *R. solanacearum* are quite great (Elphistone, 2005). In Indonesia, this disease causes a very big disadvantage and the disease intensity can reach 75% (Fortman & Martin, 1998).

The disadvantage due to this disease is increasingly high for in the field it commonly attacks chili joint together and synergic. Numerous attempts to overcome the main disease of being wilt disease on chili have been sought to, but an effective and efficient controlling method has not been discovered. It is because both pathogens can withstand in the soil and both of them synergize to attack chili. Therefore, an attempt to overcome the pathogen attack and to improve the chili resistance is necessary.

Biological control can improve the result by suppressing pathogen inoculum, protecting the plant against infection, and improving the plant resistance from pathogen. Microorganism will block pathogen at the root. Useful biological control with microorganism can induce root growth will be increasingly performed (Podile & Kishore, 2007).

Among Plant Growth Promoting Rhizobacteria (PGPR), fluorescent pseudomonads gain more attention. Certain strain from fluorescent pseudomonads shows its ability in suppressing the development of several plant diseases due to pathogens and inducing plant growth (Raaijmakers *et al.,* 1995). Primary mechanism of suppressing disease by biological agents are including antagonism and or inducing plant resistance (Hyakumachi, 2013). Bacteria are able to induce plant systemic resistance by producing phytohormone, solubilizing inorganiz phosphate, improving fastening of Fe and siderophore (Podile & Kishore, 2007). As suggested by Pinton *et al*. (2001), rhizobacteria is also able to induce plant growth by producing growth regulator such as auxin derivatives. Most of *Pseudomonas*, *Bacillus, Azotobacter* rhizobacteria produce growth inducing compound such as Indol Acetic Acid (IAA), gibberellins, and other substances such as cytokinin (Brimecombe *et al.*, 2001). Antibiotics produced by fluorescent pseudomonads contributing in biological control are phenazines, 2.4-diacetylphloroglucinol, pyoluteorin, pyrrolnitrin, lipopeptides, and hydrogen cyanide (Haas and Keel, 2003). Antibiosis is activity of response generally from several biological agents such as *Pseudomonas* spp, *Bacillus* spp, *Streptomyces* spp, and *Trichoderma* spp. (Alabouvette *et al.,* 2006).

Wuryandari *et al*., (2007) showed that fluorescent Pseudomonad of Pf-122 isolate can inhibit bacterial wilt disease caused by *Ralstonia solanacearum*. In the last observation (30 days) the disease index is only 49.9% at greenhouse scale. To determine the ability of fluorescent Pseudomonad to improve the plant resistance toward 2 pathogens working in synergy in the field (*R.*

solanacearum and *F. oxysporum*), research needs to be done by using fluorescent pseudomonad to induce chili plant resistance toward the primary wilt disease due to *R. solanacearum* and *F. oxysporum* in the field.

METHODOLOGY

The study is conducted in land commonly used for *Solanaceae* plant cultivation and frequently exhibited the symptom of wilt disease, which is in Lebo village, Sukodono, Sidoarjo, East Java, Indonesia. The study employs Completely Randomized Design comprising 3 treatments. The three treatments are the types of fluorescent pseudomonads isolate which is Pf-122 isolate, Pf-160 isolate, and Pf-B isolate. Each treatment has three repetitions. Control treatment as the comparator is chili without treatment with fluorescent pseudomonads

The root of chili seeds aged 30 days are immersed into fluorescent pseudomonads suspension for 30 minutes, then it is planted. Negative comparator consists of chili seed immersed into sterile water for 30 minutes and is planted in the soil. The concentration of fluorescents pseudomonads used is 10^{10} cfu/ml. Each treatment is repeated three times. To identify plant resistance toward wilt disease due to *R. solanacearum* and *F. oxysporum*, observation parameter includes incubation period, disease index, and the color change of stem tissue.

Incubation period is observed from pathogen inoculation until the appearance of wilt disease symptom on chili. The disease index is observed in the development of wilt disease symptom 5 days / plant until 30th day after inoculation. For the color change of stem tissue is observed at the 30th day, by splitting length wise the stem and measuring the length of color change in vascular tissue.

The weight of attack is estimated according to the following scale:

0 = no symptom, 1 = 1 to 10% wilt leaves, 2 = 11 to 30% wilt leaves, 3 = 31 to 60% wilt leaves, 4 = 61 to 99 % wilt leaves, and 5 = 100% wilt leaves

The magnitude of disease index is estimated by using a formula (Arwiyanto, 1995):

$$I = \frac{\sum_{i=0}^{k} k.nk}{ZxN} \times 100\%$$

The formula description is as follows; I = disease index, nk = the number of plant having disease symptom with scale k (0, 1, 2, 3, 4, 5), N = total number of inoculated plant, and Z = highest category of attack.

RESULTS AND DISCUSSION

a. Incubation period

If it is seen from the results of observation on incubation period of wilt disease, the three fluorescent pseudomonads show different result. Incubation period of wilt disease is fastest in the treatment of Pf-B isolate then in Pf-122 isolate and Pf-160 isolate which are 7 days and 8 days. Control in incubation period is 5 days. If compared to control then the treatment with Pf-122 isolate and Pf-160 isolate it is able to delay the emergence of the symptom for 3 days, whereas with Pf-B isolate it is only 2 days (Figure 1).

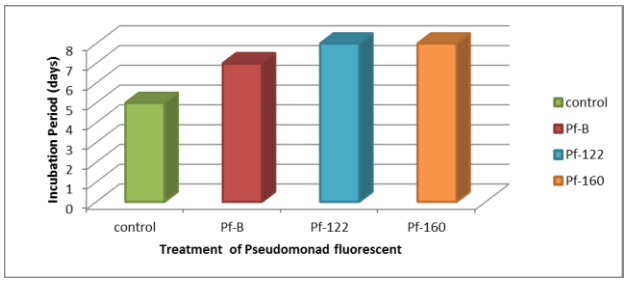

Figure-1. Incubation period of wilt disease symptoms on chilli

Short delay of incubation period of wilt disease by fluorescent pseudomonads rhizobacteria, it is possible due to lack of fluorescent pseudomonads population, which may inhibit pathogen attack. The dose of pseudomonad fluorescents rhizobacteria used to plant submergence is probably still less high, so that it is not quite able maximally in inhibiting pathogen to infect chili. The result of a study conducted Wuryandari *et al*. 2012, that chili treated with fluorescent pseudomonads of Pf-160 isolate, Pf-B isolate and Pf-122 isolate and is inoculated with *F. oxysporum*, able to delay the disease symptom longer which is 13 days, 12 days, and 6 days. The difference of incubation period delay is possible because beside pathogen which has to be inhibited is not only one, but also because different application scale. Previous research application is in greenhouse, whereas this study is conducted in the field. Field condition with more complex microorganism so that it can influence the ability of pseudomonad fluorescents in expressing the ability in producing inhibiting compound to resist pathogen and the ability in producing growth hormone for chili. Biological control of pathogen by rhizobacteria can be based on bacteria metabolite such as siderophores, antibiotics, and hydrogen cyanide. Rhizobacteria can also induce the plant growth by producing growth regulator such as auxin derivatives (Pinton *et al*, 2001).

b. Disease Index

The development of disease stating from inoculation until 30th day of observation after inoculation can be seen in Figure 2.

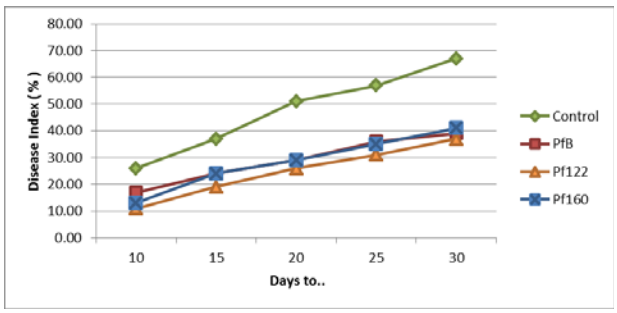

Figure-2. The development of wilt disease on chili after treated with fluorescent pseudomonads rhizobacteria

The result of disease index from the appearance of wilt disease symptom to the 30th day of observation, it is seen that the development of wilt disease on plant with fluorescent pseudomonads from the three isolates is slower

compared to control. Based on the three fluorescent pseudomonads rhizobacteria, Pf-122 isolate shows the slowest or the lowest disease development compared to other isolate fluorescent pseudomonads.

In the last observation, the treatment with Pf-122 isolate shows the lowest disease index which is 37% then it is followed by Pf-8 isolate and Pf-160 isolate which are 39% and 41%, whereas in the control it reaches 67%. If it is compared to control, then the submergence by Pf-122 isolate is able to suppress the disease development by 30%, whereas it is with Pf-B and Pf-160 isolates only able to suppress the disease development by 28% and 18% (Table 1)

Table-1. The index of wilt disease on chili with treatment using fluorescent pseudomonads rhizobacteria at 30th day

Number	Treatment	Disease Index on day 30
1	Fluorescent pseudomonads of Pf-122 isolate	37 a
2	Fluorescent pseudomonads of Pf-160 isolate	41 a
3	Fluorescent pseudomonads of Pf-B isolate	39 a
4	Control	67 b

The result shows that the application of fluorescent pseudomonads can suppress the wilt disease by *Fusarium* and *Ralstonia solanacearum*. Fluorescent pseudomonads of Pf-122 isolate is the best in suppressing the wilt disease. It is possible because fluorescent pseudomonads is able to produce a compound which can inhibit pathogens of *Fusarium* and *Ralstonia solanacearum* and can induce chili growth so that it can be resistant to the pathogen attack. Primary mechanism in suppressing disease by biological agents includes antagonism and or inducing plant resistance (Hyakumachi, 2013).

The application of fluorescent pseudomonads in chili rooting, it probably causes fluorescent pseudomonads colonize root area so that it inhibits the pathogen population causing wilt disease. Mechanism of biological control of pathogens by rhizobacteria may be in form of bacteria metabolite production. Bacteria of *Pseudomonas* genus also produces antimicrobial compound such as phenazine, pyrrolnitin, pyluteorin, phloroglucinols, cyclic lipopeptides, and hydrogen cyanide (Haas & Defago, 2005). Application of pseudomonad fluorescens also allows the plant growth to be induced so that it can be more resistant to pathogen attack of *Fusarium* and *Ralstonia solanacearum*. As suggested by Pinton *et al*., (2001), rhizobacteria is also able to induce plant growth by producing growth regulator such as auxin derivatives. Most of *Pseudomonas*, *Bacillus, Azotobacter* rhizobacteria produce growth inducing compound such as Indol Acetic Acid (IAA), gibberellins, and other substances such as cytokinin (Brimecombe *et al.*, 2001). The sufficient existence of fluorescent pseudomonads in rooting area is allegedly able to dissolve phosphate plays a role in repairing the plant. In accordance to Beattie (2007), one way to repair phosphorus deficiency on plant is by soil inoculation with phosphate solubilizing microorganism. Several saprophytic bacteria playing role as biological agents of wilt disease control is fluorescent pseudomonads bacteria on tomato, potato, and eggplant, and on chili (Saddler, 2005). Bacteria are able to induce plant systemic resistance by producing phytohormone, solubilizing inorganiz phosphate, improving fastening of Fe and siderophore (Podile & Kishore, 2007; Haas & Defago, 2005).

c. Color change on stem tissue

Based on the observation result on symptom of color change in stem vascular tissue, it is seen that the average symptom of color change in stem with the treatment using fluorescent pseudomonads, is smaller than control. Application using Pf-160 isolate shows the smallest change of color then followed by Pf-122 and Pf-B isolates (Figure 3). It is probably that fluorescent pseudomonads rhizobacteria applied is able to colonize in chili root so that it can inhibit microorganism in soil including pathogen causing wilt disease of *R. solanacearum* and *Fusarium* sp. The ability of pseudomonad fluorescents rhizobacteria of Pf-160 isolate in inhibiting disease is seemingly better compared to other fluorescent pseudomonads rhizobacteria. It is seen from the slow or small vascular tissue in the stem. Although fluorescent pseudomonads of Pf-160 isolate is better in inhibiting the damage on vascular tissue, it is apparently not different from other isolate fluorescent pseudomonads in arising symptom of wilt disease on plant. According to Baharuddin *et al* (2005), the result of his study explains that *Pseudomonas* spp. fluorescent group in plant rooting is able to colonize well so that it can suppress the attack by *R. solanacearum*. The application of fluorescent pseudomonads expected to be able to inhibit pathogens causing wilt disease which is *R. solanacearum* and *Fusarium* sp. living in rooting area before the pathogens entering the plant root tissue.

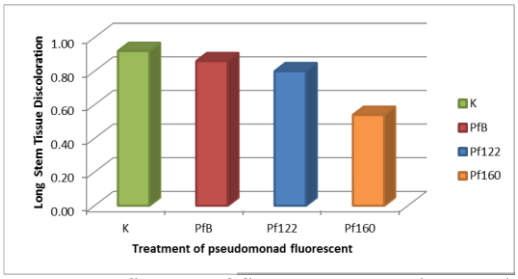

Figure-3. Influence of fluorescent pseudomonads treatment on symptom length of color on stem

CONCLUSION

Application with pseudomonad fluorescent of Pf-122 isolate compared to control is able to postpone the emergence of the symptom for 3 days. However, if it is seen from the disease index, compared to other isolate and control, Pf-122 isolate shows the highest inhibiting ability.

ACKNOWLEDGEMENT

The author would like to thank Director of Research and Public Service, Directorate-Generals of Higher Education, Ministry of Education and Culture of

Republic of Indonesia for funding this research through National Strategic Research Grant No. 180 / SP2H / PL / Dit.Littabmas /V/2013.

REFERENCES

Alabouvette C, Olivain C, and Steinberg C. Biological control of Plant Disease : the European situation. European Journal of Plant Pathology. 114 : 329 – 341.

Arwiyanto T and Hartana I. 1999. Biological Control of Tobacco Bacterial Wilt Disease: 2. Experiments in Greenhouse. J. Plant Protection Indonesia.5 (1) 50-99.

Beattie G A. 2007. Plant-Associated Bacteria: Survey, molecular phylogeny-genomics and Recent Advances. In : Gnanamanickam, S.S. (ed.). Plant-Associated Bacteria. Springer The Netherlands

Brimecombe M J, De Leij F A, and Lynch J. 2001. The Effect of Root Exudates on Rhizosphere Microbiae Populations. In: Pinton, R., Varanini, Z., Nanipieri, P. The Rhizophere. Biochemistry and Organic Substances at the Soil-Plant Interface. Marcel Dekker, Inc. New York – Basel.H. 95-140.

Elphinstone J G. 2005. The Current Bacterial Wilt Situation: A Global Overview. p. 9-28. In : Allen C, Prior P, Hayward A C. Bacterial Wilt Disease and the *Ralstonia solanacearum* Species Complex. The American Phytopathological Society Press.

Haas D and Defago G. 2005. Biological Control of Soil Borne Pathogens by fluorescent pseudomonads. Nat Rev Microbial. 3.307-319.

Fortman B A and Martin S B. 1998. Disease Management Strategies for Control of Bacterial Wilt of Tobacco in the Southeastern USA. p. 394-402. In: Prior P H, Allen C and Elphinstone J (eds.). *Bacterial Wilt Disease Molecular and Ecological Aspects*. Springer-Verlag Belin Heidelberg, INRA Paris.

Haas D And Keel C. 2003. Regulation of Antibiotic Production in Root-Colonizing Pseudomonas spp. And Relevance for Biological Control of Plant Disease. Annual Review of Phytopathology. 41, ProQuest p: 117 – 153.

Hyakumachi M. 2013. Research on Biological Control of Plant Diseases : Present state and Perspectives. J Gen Plant Pathol 79: 435 – 440.

Pinton R, Varanini Z, and Nanipieri P. 2001. The Rhizophere as a site of Biochemical Interactions Among Soil Components, Plants, and Microorganisms. In: Pinton, R., Varanini, Z., Paulo Nanipieri, P.. The Rhizophere. Biochemistry and Organic Substances at the Soil-Plant Interface. Marcel Dekker, Inc. New York–Basel. P. 1 – 18

Podile A R and Kishore G K. 2007. Plant Growth-Promoting Rhizobacteria.p. 195-230. In : Nanamanickam, S.S. (ed.). Plan-Associated Bacteria. Springer

Raaijmakers J M, Leeman M, Van Oorschot M M P, Van der Sluis I, Schipper B and Bakker P A H M. 1995. Dose-response relationships in biological control of fusarium wilt of radish by *Pseudomonas* spp. *Phytopathology* 85: 1075-1081.

Saddler G S. 2005. Management of Bacterial Wilt Disease. p.121-132. In: In : Allen, C., Prior, P., Hayward, A.C. Bacterial Wilt Disease and the *Ralstonia solanacearum* Species Complex. The American Phytopathological Society Press.

Soelaiman V and Ernawati A. 2013. Growth and Development of Chilli (*Capsicum annuum* L.) in *in vitro* at some concentrations of BAP and IAA. Bul. Agrohorti 1(1): 62 - 66.

Wuryandari Y, Purnawati A, Arwiyanto T, and Hadisutrisno B. 2007. Effect of pseudomonads fluorescens to development of wilt disease due to *Ralstonia solanacearum* in Greenhouse. Journal Saintek Kopertis Region VII. East Java. 4 (2): 48-52.

Wuryandari Y, Wiyatiningsih S, and Sulistyono. 2012. Control of *Fusarium oxysporum* wilt disease using Pseudomonas fluorescens Rhizobacteria. Proceedings of the National Seminar PERHORTI. p.507-512.