

## **Ecobiology of Bacterial Wilt of Physic Nut in Indonesia** ***Ekobiologi Layu Bakteri pada Jarak Pagar di Indonesia***

**Titiek Yulianti**

Indonesian Research Institute for Sweetener and Fiber Crops

Jln. Raya Karangploso, PO Box 199, Malang, Indonesia

E-mail: tyuliant@gmail.com

*Submitted: 21 November 2014; revised: 20 April 2015; accepted: 25 September 2015*

### **ABSTRAK**

Budi daya jarak pagar (*Jatropha curcas*) dengan sistem monokultur pada hamparan yang luas telah menimbulkan ledakan suatu penyakit. Layu bakteri yang disebabkan oleh *Ralstonia solanacearum* merupakan salah satu penyakit utama jarak pagar dan ditemukan di beberapa daerah pengembangan. Gejala yang terlihat pada tanaman yang terinfeksi adalah layu dan daun menguning sebelum waktunya atau daun layu tanpa adanya perubahan warna dan masih melekat di batang. Jaringan pembuluh berubah warna kecokelatan. Akar utama dan sekunder busuk berwarna cokelat kehitaman. Pada tanaman yang terinfeksi cukup berat, daun-daunnya akan gugur, bagian batang menjadi cokelat, dan akhirnya tanaman mati. Berdasar reaksi oksidasi sumber gula, biovar *R. solanacearum* yang diisolasi dari tanaman jarak pagar dari Malang, Jawa Timur mirip dengan biovar 5, sedangkan yang dari Pati, Jawa Tengah, berbeda dengan biovar standar yang ada. Bakteri ini menginfeksi tomat, cabai merah, dan terong, tetapi tidak menginfeksi tembakau ataupun jagung. Observasi lapangan untuk mengetahui perkembangan layu bakteri pada tanaman jarak pagar dan penyebarannya menunjukkan bahwa fluktuasi kejadian penyakit berkorelasi positif dengan curah hujan. Streptomycin sulfat atau kombinasi beberapa jenis antagonis merupakan cara pengendalian yang baik. Selain itu, mengoleskan CaCO<sub>3</sub> pada luka akibat pemangkasan dapat mencegah penyakit berkembang lebih lanjut. Arah penelitian ke depan untuk pengendalian penyakit ini adalah pengendalian terpadu yang menitikberatkan kepada pertanian dan lingkungan yang keberlanjutan, misalnya penambahan antagonis, mikroorganisme berguna, bahan organik, serta pemupukan seimbang.

Kata kunci: *Ralstonia solanacearum*, jarak pagar, Indonesia, penyakit

### **ABSTRACT**

Growing physic nut (*Jatropha curcas*) under monoculture system in large areas has generated disease outbreak. Bacterial wilt caused by *Ralstonia solanacearum* is one of the major diseases found in several regions. The symptom of the infected plant is wilting and premature leaf yellowing or leaves wilting without changing colour and still attaching to the stem. The vascular tissues show a brown discoloration. The primary and secondary roots may become brown to black. Severe infection causes leaves of diseased plant to fall, the stem to become brown and eventually death. Based on oxidation reaction of sugar source the biovar of *R. solanacearum* isolated from physic nut in Malang (East Java) was similar to biovar 5, but isolate from Pati, Central Java was different from the standard biovar. The pathogen infected tomato, red chili, and egg plant but not tobacco or maize. A field observation to determine the development of bacterial wilt in physic nut and its spread pattern demonstrated that disease fluctuation incidence was positively correlated to rainfall. Streptomycin sulphate or combination of antagonists gave a good disease control. Furthermore, smearing CaCO<sub>3</sub> on wound caused by pruning could prevent disease development. The best control measure is integration of several control measures which encourage sustainable agriculture and environment, including the addition of antagonists, effective microorganism, organic matter, and balanced fertilizer.

Keywords: *Ralstonia solanacearum*, physic nut, Indonesia, disease

## INTRODUCTION

Currently, development of physic nut (*Jatropha curcas*) has been encouraged by the government of Indonesia since 2005 when an issue spread out that reserve of fuel has been depleted. Physic nut is promising source for biofuel or biodiesel and considered as renewable energy options in the future. Physic nut is mainly developed in marginal lands without irrigation, as Allorerung *et al.* (2006) noted that the plant could grow in drought climate and marginal land, but it needs sufficient water and nutrition for its optimum production. Mulyani *et al.* (2006) reported that 12.4 millions hectares of an unused land; 3.1 millions hectares of savanna; and around 1 million hectares of blady grass (*Imperata cylindrical*) land within 13 provinces were potentially suited for physic nut development.

Although the plant is claimed resistant to pests and diseases, growing in large areas will create such problem (Prihandana & Hendroko 2006). Several diseases such as bacterial wilt, charcoal rot, bacterial blight, Fusarium wilt, anthracnose, leaf spots, and mildew have been reported in some developed areas (Yulianti *et al.* 2007; Hartati *et al.* 2008.; Ginting & Maryono 2009; Hendra 2009; Laksono *et al.* 2010). Based on our observation, bacterial wilt caused by *Ralstonia solanacearum* (Smith) Yabuuchi (Yabuuchi *et al.* 1995) considered as major disease of physic nut since it mild-severely occurred in Lampung, Central Java, and West Java. According to Ginting & Maryono (2009), disease severity of bacterial blight in Lampung ranged from 9–32%. Although, the spread of *R. solanacearum* is generally very restricted and slow, Karmawati & Sukanto (2009) found that bacterial wilt rapidly spread and caused around 1% nursery and young plants died within three weeks of 10 hectares area in the nursery garden of Center of Cikarang. This incidence showed that the disease was potentially major constrain in physics nuts development. There

was no estimation of yield loss; however, severe incidence of bacterial blight could kill physic nut plants of more than 50%.

*R. solanacearum* has more than 200 host plant species of more than 50 botanical families including weeds (Meng 2013). The pathogen is soil borne and could survive for varying periods of time (days to years) in moist soil and in irrigation (drainage) water, even in >75 cm of soil depth depends on temperature, pH, soil moisture, organic matter content, and the presence of microbial antagonists (Olson 2005).

Physic nut grows well and gives high seed yield in light soil (sandy clay texture) (Okabe & Somabhi 1989), whilst the pathogen is well adapted in the same type of soil texture and warm temperature (24–35°C). This means, the plant will severely suffer from the disease, if inoculum of *R. solanacearum* has been established in the soil.

*R. solanacearum* has long been known as the main causal agent of tropical plants disease. Therefore, bacterial wilt outbreak will difficult to control. The cheapest way to manage the disease in other crops is prevent the outbreak by using resistant variety. However, the stability of resistance several crops is highly affected by pathogen density, pathogen strains, and several soil factors. Cultural, chemical, and biological method such as sanitation, improve soil ecosystem balance, and crop rotation were reported gave unsatisfied result. Ahmed *et al.* (2013) reported that *R. solanacearum* in potato was not effectively control by cultural, chemical, and biological measures due to wide host range and genetic diversity of the pathogen.

This paper review ecobiology of the bacterial wilt of physic nut in Indonesia, several attempts to control the disease in physic nut under trial scales, other possible control measures to manage the disease based on other crop issues, and future direction for research and development of the disease.

## DISEASE SYMPTOMS OF BACTERIAL WILT (*Ralstonia solanacearum*) OF PHYSIC NUT

The main symptom of the infected physic nut plant is wilting and premature leaf yellowing. Sometimes, one side of the plant show wilting or rotting symptoms starting from stem above the soil line (Figure 1a). In other cases, leaves wilt without changing colour and stay attached to the stem (Figure 1b). The vascular tissues show a brown discoloration when cut open (Figure 1c). The primary and secondary roots may become brown to black. Severe infection causes leaves of diseased plant fall, the stem becomes brown, and eventually die. When the plant died, the bacteria spread out of the xylem and returned to the soil through the roots. They survived persist in the environment until next host becomes available (Dalsing & Allen 2014).

A white, slimy mass of bacteria exudes from vascular bundles, when broken or cut. This slime oozes spontaneously from the cut surface of the stem in the form of threads, when suspended in water (Figure 1d).

## PHYSIOLOGICAL CHARACTERISTICS OF *RALSTONIA SOLANACEARUM* OF PHYSIC NUT

*Ralstonia solanacearum* is a Gram-negative, rod-shaped, strictly aerobic bacterium

that is  $0.5\text{--}0.7 \times 1.5\text{--}2.0 \mu\text{m}$  in size with the optimal growth temperature is  $28\text{--}32^\circ\text{C}$ . The virulent type colonies appear after 36–38 hours at  $28^\circ\text{C}$  as white or cream-colored, irregularly shaped, highly fluidal, and opaque when grown in soil medium. Isolation from symptomatic plant used Kelman's tetrazolium (TZC) medium produced white slimy colonies with red in the centre (Figure 2a) or murky slimy colonies when cultured in CPG (Figure 2b) (personal collection). The TZC medium was used to differentiate between virulent and non-virulent colony types. As Kelman (1954) reported, virulent colonies looked white with pink centers whilst non-virulent colonies were dark red.

Based on oxidation reaction of sugar source tested at laboratorium of Phytopathology-IRISFC, *R. solanacearum* isolated from Pati, Central Java used maltose, lactose, cellobiose, mannitol, and sorbitol, except dulcitol.

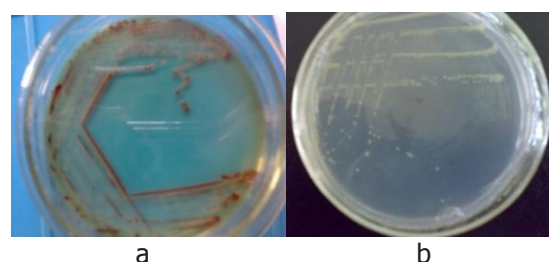


Figure 2. Colonies of *Ralstonia solanacearum* on TZC medium (a) and on CPG medium (b)

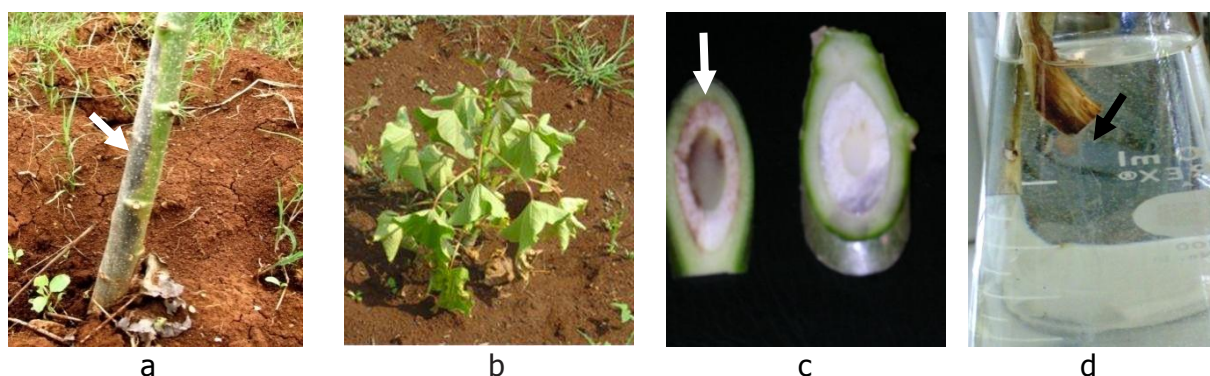
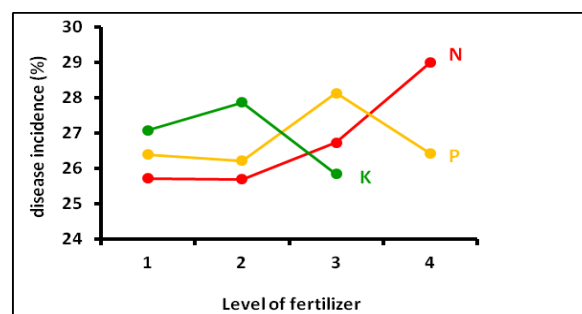


Figure 1. Symptoms of bacterial wilt on physic nut (a, b, c) and bacterial masses exude from infected plant and inside vascular tissue (d)

In general, cultural methods; genetic variation of host plant; and environmental factors such as soil, temperature, humidity, and rainfall highly affect the development of bacterial wilt (Supriadi *et al.* 2001). Bacterial wilt in physic nut was relatively new and there was no information regarding epidemiology of the disease. Yulianti & Hidayah (2010) showed that bacterial wilt severity on the physic nut was in line with rainfall; higher severity occurred at peak rainy season. This is in agreement with Janse (2004) and Nesmith & Jenkins (1985) that the most favorable for development of *R. solanacearum* is at rainy season. Furthermore, the spread pattern was irregular but tended to develop spotted around infected plants showing that it was disseminated by soil from diseased roots to healthy ones. The severe the disease, the quicker it is to spread and develop. In addition, the suffered plants were hardly to recover (Yulianti & Hidayah 2010). As Champoiseau *et al.* (2009) described, the bacteria spread from plant to plant when bacteria moved from infected roots to nearby healthy plants roots. It could be also disseminated by soil transfer on machinery and surface run off water after irrigation or rainfall (Coutinho 2005).

Fertilizer or other soil amendment affected disease development differently. Hidayah & Yulianti (2008) observed bacterial wilt disease incidence on fertilization trial plots. The observation was done every two weeks and terminated when the disease stopped developing. It was found that neither single nor in combination of N, P, or K fertilizers gave significant effect on the disease development, but there was a tendency that high nitrogen increased disease incidence and giving higher dosage of potassium decreased it (Figure 3). Dalsing & Allen (2014) revealed that nitrogen promoted *R. solanacearum* virulence through nitrate assimilation. The assimilation enhanced bacteria attached root, helped initial stage of infection, and controlled extracellular polysaccharide (EPS) production. EPS

is a major bacterial wilt virulence factor by blocking xylem vessels (Genin & Denny, 2012). Messiha *et al.* (2007) found that mechanism of disease suppression of *R. solanacearum* by means of NPK amendment varied among to the soil type. NPK fertilization reduced bacterial wilt in Egyptian sandy and clay soils but not in Dutch clay soils.



\*) Level of fertilizer: N1= 0, N2= 22.5, N3= 45, N4= 90 kg N/ha; P1= 0, P2= 18, P3= 36, P4= 72 kg P/ha; K1= 0, K2= 30, K3= 60 kg K/ha

Figure 3. Trend of disease incidence of bacterial wilt in physic nut treated with several level of N, P, K fertilizer

## CONTROL OF THE DISEASE

There are several general strategies to control bacterial wilt includes the use of resistant varieties, cultural, chemical, biofumi-gants, and biological control (Hsu 1991).

Cultural control for *R. solanacearum* includes nonhost crop rotation, soil amendment, the use disease free plant materials, is the most popular method control. This method aims to reduce initial inoculums. Deberdt *et al.* (2015) found that the proportion of infected plants and bacterial wilt incidence in tomato decreased by 86% and 60% when rotated with *Raphanus sativus* cv. Melody and *Crotalaria spectabilis*. Crop rotation for perennial crops such physic nut is impracticable; thus intercropping system with non host or toxic crops is another potential alternative choice. Ardhana (2008) reported that *R. solanacearum* of physic nuts infected tomato (*Lycopersicon esculentum*), red chili (*Capsicum*

*annuum*), egg plant (*Solanum melongena*) but it did not infect tobacco (*Nicotiana tabacum*), peanut (*Arachis hypogaea*), ginger (*Zingiber officinale*), or maize (*Zea mays*). *Brassicaceae* such mustard (*Brassica sinensis*), cabbage (*B. oleracea*), and Broccoli (*B. oleracea*) had been tried as source of biofumigant for *R. solanacearum* in tobacco (Yulianti 2008). The reduction of *R. solanacearum* population since isothiocyanates (ITC), released from shredded leaves when the *Brassicaceae* was incorporated into the soil, was toxic to the pathogen. Composted the *Brassicaceae* residues also enhanced soil microbial population which then suppressed pathogen population. Supriadi *et al.* (2001) and Hsu (1991) suggested that grafting on resistant rootstocks or disinfection of plant materials could be used to inhibit disease development.

The pathogen usually infected plants by injured roots, stem wounds, or through stomata. Fortnum & Kluepfel (2005) also found that pruning process of tobacco leaves increased disease incidence since the bacteria entered into plants through the wound. Hence, the use of calcium carbonate ( $\text{CaCO}_3$ ) coating on wounded stalk due to pruning reduced disease incidence of bacterial wilt of physic nut up to 50% (Hidayah & Yulianti 2010). He *et al.* (2014) obtained that when  $\text{Ca}^{2+}$  concentration in plant tissue increased, pectinase activity of *R. solanacearum* decreased following reduced bacterial wilt disease incidence. In addition, application of organic fertilizer and  $\text{CaCO}_3$  increased soil pH and lowered the population of *R. solanacearum* almost 100 times compared to control. Jiang *et al.* (2013) obtained that increase the addition of calcium from 0.5 mM to 25 mM into 3 l nutrient solution for tomato hydroponic reduced disease severity up to 43%. The reduction of disease severity was due to the increased of  $\text{H}_2\text{O}_2$ , which was stimulated by calcium. When plants are under biotic or environmental stresses, they usually generate reactive oxygen species (ROS) i.e.  $\text{H}_2\text{O}_2$  which has a bactericidal activity. Calcium also enhanced the

generation rate of polyphenol oxidase (PPO) and peroxidase (POD). Both PPO and POD have an important role in plant disease resistance to pathogens. Yamazaki & Hoshina (1995) also reported that calcium increased resistance of tomato to bacterial wilt and decrease the population of pathogen in the xylem.

Biological control is an environmentally friendly alternative control measure and could be integrated with other control practices for effective disease management. Biological control for *R. solanacearum* using beneficial microorganisms such antagonists, bacteriophage, effective microorganisms (EM), or other bioorganic fertilizer has been reported in other crops (Yamada 2012; Lwin & Ranamukhaarachchi 2006; Ding *et al.* 2013). Antagonist, a microorganism that suppresses the pathogen (Pal & Gardener 2006), and bacteriophage, virus that specifically kill bacteria (Frampton *et al.* 2012) are biocontrol agent have direct antagonism on the pathogen. EM and bioorganic fertilizer usually contained several mixtures of beneficial bacteria or fungi that have function on plant growth support. They sometimes have antagonist capability or just acted as competitor. However, information of the use of biocontrol agent for *R. solanacearum* in physic nut little has been very limited. When young jatropha plants were severely suffered from *R. solanacearum* infection in Cikarang (West Java) Nursery Garden, Karmawati & Sukanto (2009) tested several antagonists of *R. solanacearum* of physic nut from Research Institute for Spices and Medicinal Plants (Balitro) and Ornamental Plants research Center (Balithi) collections. They reported that a mixture of *Trichoderma lactae* and *Bacillus pantotkenticus*, and a mixture of *Bacillus* sp. and *Pseudomonas fluorescens* did not affect the disease development, and yet combination of 5 species of *B. subtilis*, *B. laterosporus*, *B. licheniformis*, *B. megaterium*, *B. pumilus*, and 4 species of *Trichoderma* (*T. harzianum*, *T. viride*, *T. koningii*, and *T. polysporum*) inhibited the disease development as

good as the application of streptomycin sulphate 20%.

### **FUTURE DIRECTION FOR BACTERIAL WILT OF PHYSIC NUTS RESEARCH AND DEVELOPMENT**

Several components of bacterial wilt control of other crops have been issued, but on *Jatropha* have not been extensively done. Considering bacterial wilt as a potential threat of *Jatropha* cultivation, research on the management of the disease is urgently needed. The best approach is the method should provide higher growth and production, whilst environmentally friendly manage the disease.

Although physic nut can lives in marginal land, it will grow better under appropriate maintenance including irrigation, the addition of effective microorganism (EM), organic matter, and balance fertilizer. Improvement of plant growth performance could increase plant defense ability against pathogen infection directly or indirectly. Djajadi *et al.* (2010) found that irrigation with 7 days interval enhanced the growth of physic nut. Inoculation of EM into soil would alter soil microbiological equilibrium, improve soil quality, enhance crop production and protection (Higa & Parr 1994). Lwin & Ranamukhaarachchi (2006) obtained good result when used either EM or Bokashi to control *R. solanacearum* on tomato. They found that some bacteria isolated from Bokashi or EM were antagonistic to *R. solanacearum*. According to Hussain *et al.* (1993) EM contained a mixture of photosynthetic bacteria, *Azotobacter*, *Streptomyces*, and *Lactobacillus* spp. Hence, EM improved crop yield by accelerating nitrogen fixation, decomposition of organic material in the soil, controlling soil diseases, and increasing photosynthesis. Furthermore, the beneficial microorganisms can fix atmospheric nitrogen, decompose organic wastes and residues, detoxify pesticides, suppress plant diseases and soil-borne pathogens, enhance nutrient cy-

cling, and produce bioactive compounds such as vitamins, hormones, and enzymes that stimulate plant grow.

Various arbuscular mycorrhizal fungi (AMF) colonizes root tips of physic nut (Muzakkir 2010; Charoenpakdee *et al.* 2010) and improves the nutritional absorption (Muzakkir 2010), promotes the root growth and dry weight of the plant (Irianto 2009; Saodah *et al.* 2010), development of seedlings, and enhance the stress tolerance capacity of physic nut (She *et al.* 2012). The role of AMF as biocontrol agent on physic nut has not been explored, although there were several reports on other crops. Hence, evaluation of AMF isolated from indigenous *Jatropha* for control of bacterial wilt should be conducted. Monther *et al.* (2012) reported that inoculation of *Glomus mosseae* reduced bacterial wilt infection in tomato, and yet enhanced shoot weight and root morphology (tips, length, surface area volume, fresh weight, and dry weight). Colonization of hyphal net of AMF in roots facilitates roots absorb more nutrient and also modifies the root cortex cells and root structure which helps the plant to prevent *R. solanacearum* infection. Zhu & Yao (2004) found that AMF (*G. versiforme*) induced phenols production locally or systemically on tomato plant causing inhibition of *R. solanacearum* infection and decrease the pathogen population.

Ayana *et al.* (2011) reported that soil amendment with silicon fertilizer or sugarcane bagasse reduced *R. solanacearum* population, bacterial wilt incidence, index of disease severity in moderately resistant tomato. Thus, introducing potential AMF combined with biofertilizer which could act as antagonist and Si amendment in infested soil could also be evaluated as integrated management of bacterial wilt. It could be expected that the combination treatment will increase physic nut yield and increase the level of plant resistance to pathogen, especially *R. solanacearum*.

## REFERENCES

- Ahmed, NN, Islam, MR, Hossain, MA, Meah, MB & Hossain, MM 2013, Determination of races and biovars of *Ralstonia solanacearum* causing bacterial wilt disease of potato, *Journal of Agricultural Science*, 5(6):86–93, doi:10.5539/jas.v5n6p86 URL: <http://dx.doi.org/10.5539/jas.v5n6p86>.
- Allorerung, D, Mahmud, Z, Rivaie, AA, Effendi, DS & Mulyani, A 2006, Peta kesesuaian lahan dan iklim jarak pagar (*Jatropha curcas* L.), Makalah disampaikan pada Lokakarya Status Teknologi Budi Daya Jarak Pagar (*Jatropha curcas* L.), Badan Penelitian dan Pengembangan Pertanian, Jakarta, 11–12 April 2006, 14 hlm.
- Ardhana, R 2008, Patogenisitas *Ralstonia solanacearum* isolate asal jarak pagar (*Jatropha curcas*) terhadap beberapa tanaman, Skripsi Sarjana Pertanian Universitas Brawijaya, 34 hlm.
- Ayana, G, Fininsa, C, Ahmed, S & Wydra, K 2011, Effects of soil amendment on bacterial wilt caused by *Ralstonia solanacearum* and tomato yields in Ethiopia, *Journal of Plant Protection Research*, 51:71–75.
- Champoiseau, PG, Jones, JB & Allen, C 2009, *Ralstonia solanacearum* race 3 biovar 2 causes tropical losses and temperate anxieties, *Online, Plant Health Progress*, doi:10.1094/PHP-2009-0313-01-RV, downloaded on 5 February 2013 (<http://www.apsnet.org/publications/apsnetfeatures/Pages/Ralstonia.aspx>).
- Charoenpakdee, S, Cherdchai, P, Dell, B & Lumyong, S 2010, The mycorrhizal status of indigenous arbuscular mycorrhizal fungi of physic nut (*Jatropha curcas*) in Thailand, *Mycosphere*, 1(2):167–181.
- Coutinho, TA 2005, Introduction and prospectus on the survival of *R. solanacearum*, in Allen, C, Prior, P & Hayward, AC editor, *Bacterial wilt disease and the Ralstonia solanacearum species complex*, APS Press, Minnesota, p. 29–38.
- Dalsing, BL & Allen, C 2014, Nitrate assimilation contributes to *Ralstonia solanacearum* root attachment, stem colonization, and virulence, *Journal of Bacteriology*, 196:949–996.
- Deberdt, P, Gozé, E, Coranson-Beaudu, R, Perrin, B, Fernandes, P, Lucas, P & Ratnadass, A 2015, *Crotalaria spectabilis* and *Raphanus sativus* as previous crops show promise for the control of bacterial wilt of tomato without reducing bacterial populations, *Journal of Phytopathology*, 163:377–385.
- Ding, C, Shen, Q, Zhang, R & Chen, W 2013, Evaluation of rhizosphere bacteria and derived bioorganic fertilizers as potential biocontrol agents against bacterial wilt (*Ralstonia solanacearum*) of potato, *Plant and Soil*, 366: 453–466.
- Djajadi, Heliyanto, B & Hidayah, N 2010, Pengaruh media tanam dan frekuensi pemberian air terhadap sifat fisik, kimia, dan biologi tanah serta pertumbuhan jarak pagar, *Jurnal Littri*, 16:64–69.
- Fortnum, BA & Kluepfel, D 2005, Mechanization has contributed to the spread of bacterial wilt on flue-cured tobacco in the Southeastern USA, in Allen, C, Prior, P & Hayward, AC editor, *Bacterial wilt disease and the Ralstonia solanacearum species complex*, APS Press, Minnesota. p. 51–59.
- Frampton, RA, Pitman, AR & Fineran, PC 2012, Advances in bacteriophage-mediated control of plant pathogens, *International Journal of Microbiology*, 2012:326452, doi: 10.1155/2012/326452.
- Genin, S & Denny, TP 2012, Pathogenomics of the *Ralstonia solanacearum* species complex. *Annu. Rev. Phytopathol.* 50:67–89, doi:10.1146/annurev-phyto-081211-173000.
- Ginting, C & Maryono, T 2009, Physic nut (*Jatropha curcas* L.) diseases in Lampung Province, *Biotropia*, 16(1):45–54.
- Hartati, SY, Wahyuno, D, Karmawati, E & Supriadi 2008, Inventarisasi dan identifikasi penyakit utama pada tanaman jarak pagar (*Jatropha curcas* L.), *Prosiding Lokakarya Nasional III Inovasi Teknologi Jarak Pagar untuk Mendukung Program Desa Mandiri Energi*, Bayu-media Publishing, Malang, ISBN: 978-602-8299-04-6, hlm. 294–301.
- He, K, Yang, SY, Li, H, Wang, H & Li, ZL 2014, Effects of calcium carbonate on the survival of *Ralstonia solanacearum* in soil and control of tobacco bacterial wilt, *European Journal of Plant Pathology*, 140:665–675 DOI:10.1007/s10658-014-0496-4.
- Hendra, J 2009, Sebaran dan tingkat keparahan hama dan penyakit pada tanaman jarak pagar di Lampung, *Prosiding Lokakarya Nasional IV Akselerasi Inovasi Teknologi Jarak Pagar Me-*

- nuju Kemandirian Energi, Surya Pena Gemilang Publishing, Malang, ISBN: 978-602-8253-17-8, hlm. 210–215.
- Hidayah, N & Yulianti, T 2008, Perkembangan penyakit layu bakteri jarak pagar (*Jatropha curcas* L.) di KP Muktiharjo, *Prosiding Lokakarya Nasional III Inovasi Teknologi Jarak Pagar untuk Mendukung Program Desa Mandiri Energi*, Bayumedia Publishing, Malang, ISBN: 978-602-8299-04-6, hlm. 308–312
- Hidayah, N & Yulianti, T 2010, Peranan bahan penutup luka pangkasan terhadap perkembangan penyakit layu bakteri, *Prosiding Lokakarya Nasional V Inovasi Teknologi dan Cluster Pioneer menuju DME Berbasis Jarak Pagar*, Tunggal Mandiri Publishing, Malang, ISBN: 978-602-8878-12-8, hlm. 222–225.
- Higa, T & Parr, J 1994, Beneficial and effective microorganisms for sustainable agriculture and environment, Atami, Japan: International Nature Farming Research Center, downloaded 12 January 2014 (<http://www.agriton.nl/higa.html>)
- Hsu, ST 1991, Ecology and control of *Pseudomonas solanacearum* in Taiwan, *Plant Protection Bulletin Taiwan*, 33:72–79.
- Hussain, T, Ahmad, R, Jillana, G, Yaseen, M & Akhtar, SN 1993, *Applied EM technology*, Nature Farm Research Center, University Agriculture, Faisalabad, Pakistan, p. 1–6.
- Irianto, RSB 2009, Pengaruh inokulasi fungi mikoriza arbuskula terhadap pertumbuhan bibit jarak pagar di persemaian, *Jurnal Penelitian Hutan dan Konservasi Alam*, 6:195–201.
- Janse, JD 2004, Diagnostic protocols for regulated pests: *Ralstonia solanacearum*, *OEPP/EPPO Bulletin*, 34:173–178.
- Jiang, JF, Li, JG & Dong, YH 2013, Effect of calcium nutrition on resistance of tomato against bacterial wilt induced by *Ralstonia solanacearum*, *European Journal of Plant Pathology*, 136:547–555, DOI 10.1007/s10658-013-0186-7.
- Karmawati, E & Sukamto 2009, Pengendalian penyakit bakteri jarak pagar dengan mikroba antagonis, *Prosiding Lokakarya Nasional IV Akselerasi Inovasi Teknologi Jarak Pagar Menuju Kemandirian Energi*, Surya Pena Gemilang Publishing, Malang, ISBN: 978-602-8253-17-8, hlm. 223–227.
- Kelman, A 1954, The relationship of pathogenicity of *Pseudomonas solanacearum* to colony appearance in a tetrazolium medium, *Phytopathology*, 44:693–695.
- Laksono, DW, Nasahi, C & Susniahi, N 2010, Inventarisasi penyakit jarak pagar (*Jatropha curcas* L.) pada tiga daerah di Jawa Barat, *Jurnal Agrikultura*, 21:31–38.
- Lwin, M & Ranamukhaarachchi, SL 2006, Development of biological control of *Ralstonia solanacearum* through antagonistic microbial populations, *International Journal of Agriculture & Biology*, 8:657–660, downloaded 11 October 2013 (<http://www.fspublishers.org>).
- Meng, F 2013, *Ralstonia solanacearum* species complex and bacterial wilt disease, *Bacteriology & Parasitology*, 4:2, downloaded 12 January 2014 (<http://dx.doi.org/10.4172/2155-9597.1000>)
- Messiha, NAS, van Bruggen, AHC, van Diepeningen, AD, de Vosm, OJ, Termorshuizen, AJ, Tjou-Tam-Sin, NNA & Janse, JD 2007, Potato brown rot incidence and severity under different management and amendment regimes in different soil types, *European Journal of Plant Pathology*, 119:367–381.
- Monther, MT, Sijam, K & Othman, R 2012, The potential of endomycorrhizal fungi in controlling tomato bacterial wilt *Ralstonia solanacearum* under glasshouse conditions, *African Journal of Biotechnology*, 11:13.085–13.094, DOI:10.5897/AJB11.3629.
- Mulyani, A, Agus, F & Allorerung, D 2006, Potensi sumber daya lahan untuk pengembangan jarak pagar (*Jatropha curcas* L.) di Indonesia, *Jurnal Penelitian dan Pengembangan Pertanian*, 25(4):130–138, downloaded 28 January 2014 ([http://www.Pustaka-deptan.go.id/publication/p3254\\_063.pdf](http://www.Pustaka-deptan.go.id/publication/p3254_063.pdf)).
- Muzakkir 2010, Keragaman dan potensi pemanfaatan fungi mikoriza arbuskula indigenus bersama pupuk hijau terhadap tanaman jarak pagar (*Jatropha curcas* L.) di lahan kritis, (Skripsi), Universitas Andalas, Padang, 210 hlm.
- Nesmith, WC & Jenkins, JrSF 1985, Influence of antagonists and controlled matric potential on the survival of *Pseudomonas solanacearum* in four North Carolina soils, *Phytopathology*, 75: 1182–1187
- Okabe, T & Somabhi, M 1989, Ecophysiological studies on drought tolerant crops suited to the Northeast Thailand, Technical Paper No. 5, Agricultural Development Research Center



- in Northeast Thailand, Moe Din Daeng, Khon Kaen 40000, Thailand.
- Olson, HA 2005, *Ralstonia solanacearum*: Pathogen profile, Department of Plant Pathology, NC State University, Raleigh, downloaded on 16 May 2009 ([http://www.cals.ncsu.edu/course/pp728/Ralstonia/Ralstonia\\_Solanacearum.html](http://www.cals.ncsu.edu/course/pp728/Ralstonia/Ralstonia_Solanacearum.html)).
- Pal, KK & Gardener, BMS 2006, *Biological control of plant pathogens the plant health instructor*, DOI: 10.1094/PHI-A-2006-1117-02, American Phytopathological Society, downloaded 23 January 2014 (<http://www.apsnet.org/edcenter/advanced/topics/Pages/BiologicalControl.aspx>).
- Prihandana, R & Hendroko, R 2006, *Petunjuk budi daya jarak pagar*, AgroMedia Pustaka, Tangerang 79 hlm.
- Saodah DW, Nuraini, A, Suherman, C 2010, The growth of the physic nut plant (*Jatropha curcas* L.) inoculated by dosages of arbuscular mycorrhizal fungi (AMF) in various level of drought stress, *Prosiding Lokakarya Nasional V Inovasi Teknologi dan Cluster Pioneer menuju DME Berbasis Jarak Pagar*, Tunggal Mandiri Publishing, Malang, ISBN: 978-602-8878-12-8, hlm. 113–123.
- She, SM, Tu, B, Liu, DJ & Yang, XH 2012, Advance and prospect of mycorrhizal physic nut, *Advance Material Research*, 518–523:5.381–5.384.
- Supriadi, Mulya, K & Sitepu, D 2001, Bacterial wilt disease of woody trees caused by *Ralstonia solanacearum*: A review, *Jurnal Litbang Pertanian*, 20(3):106–112.
- Yabuuchi E, Kosako Y, Yano I, Hotta H, Nishiuchi, Y 1995, Transfer of two *Burkholderia* and an *Alcaligenes* species to *Ralstonia* gen. nov. proposal of *Ralstonia pickettii* (Ralston, Palleroni and Doudoroff 1973) comb. nov., *Ralstonia solanacearum* (Smith 1896) comb. nov. and *Ralstonia eutropha* (Davis 1969) comb. nov. *Microbiology Immunology*, 39:897–904.
- Yamada, T 2012, Bacteriophages of *Ralstonia solanacearum*: Their diversity and utilization as biocontrol agents in agriculture in Kurtboke, I (Ed.), *Bacteriophages*, p. 113–138, downloaded 23 January 2014 ([www.intechopen.com/download/pdf/32282](http://www.intechopen.com/download/pdf/32282)).
- Yamazaki, H & Hoshina, T 1995, Calcium nutrition affects resistance of tomato seedling to bacterial wilt, *Horticultural Science*, 30:91–93.
- Yulianti, T 2008, Potency of Brassica residues as biofumigation for control of bacterial wilt of tobacco in Indonesia, Third International Biofumigation Symposium, CSIRO Canberra-Australia.
- Yulianti, T & Hidayah, N 2010, Pola sebaran penyakit layu bakteri (*Ralstonia solanacearum*) pada tanaman jarak pagar, *Prosiding Lokakarya Nasional V Inovasi Teknologi dan Cluster Pioneer menuju DME Berbasis Jarak Pagar*, Tunggal Mandiri Publishing, Malang, ISBN: 978-602-8878-12-8, hlm. 172–176.
- Yulianti, T, Suhara, C & Hidayah, N 2007, Penyakit tanaman jarak pagar (*Jatropha curcas* L.). *Prosiding Lokakarya II Status Teknologi Jarak Pagar (Jatropha curcas L.)*, Puslitbangbun Bogor. ISBN: 979-8451-45-7, hlm. 91–96.
- Zhu, HH & Yao, Q 2004, Localized and systemic increase of phenols in tomato roots induced by *Glomus versiforme* Inhibits *Ralstonia solanacearum*, *Journal of Phytopathology*, 152: 537–542, DOI: 10.1111/j.1439-0434.2004.00892.x.