### **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: tvuliant@amail.com

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#### **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 lavu dan daun menguning sebelum waktunya atau daun lavu 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-daunya 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 prunning 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

urrently, development of physic nut (*Ja-→ tropha 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 occured 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. solanacea*rum is generally very restricted and slow, Karmawati & Sukamto (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  $0.5-0.7 \times 1.5-2.0 \, \mu m$  in size with the optimal growth temperature is 28-32°C. The virulent type colonies appear after 36-38 hours at 28°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.

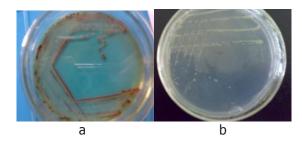


Figure 2. Colonies of *Ralstonia solanacearum* on TZC medium (a) and on CPG medium (b)







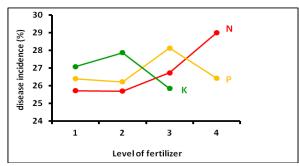


Figure 1. Symptoms of bacterial wilt on physic nut (a, b, c) and bacterial masses exude from infected plant and inside vascular tissue (d)

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, biofumigants, 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 tabaccum), peanut (Arachis hypogaea), ginger (Zingiber officinale), or maize (Zea mays). Brassicaceae such mustard (Brassica sinensis), cabbage (B. oleracea), and Brocolli (B. oleracea) had been tried as source of biofumigant for R. solanacearum in tobacco (Yulianti 2008). The reduction of R. solanacearum population sinced 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 (CaCO<sub>3</sub>) coating on wounded stalk due to prunning reduced disease incidence of bacterial wilt of physic nut up to 50% (Hidayah & Yulianti 2010). He et al. (2014) obtained that when Ca<sup>2+</sup> concentration in plant tissue increased, pectinase activity of R. solanacearum decreased following reduced bacterial wilt disease incidence. In addition, application of organic fertilizer and CaCO<sub>3</sub> 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 I nutrient solution for tomato hydroponic reduced disease severity up to 43%. The reduction of disease severity was due to the increased of H<sub>2</sub>O<sub>2</sub>, which was stimulated by calcium. When plants are underbiotic or environmental stresses, they usually generate reactive oxygen species (ROS) i.e. H<sub>2</sub>O<sub>2</sub> 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 physics nut little has been very When young jatropha plants were severely suffered from R. solanacearum infection in Cikarang (West Java) Nursery Garden, Karmawati & Sukamto (2009) tested several antagonists of R. solanacearum of physic nut from Research Institute for Spices and Medicinal Plants (Balittro) and Ornamental Plants research Center (Balithi) collections. 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. licheniformus, 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, toxify pesticides, suppress plant diseases and soil-borne pathogens, enhance nutrient cycling, 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. solanacaerum 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*.

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