

## 10. Development of component technologies for control of bacterial wilt in potato

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### Abstract

Research strategies to control potato bacterial wilt (*Ralstonia solanacearum*) were conducted in Pangalengan subdistrict in West Java from January to December 2001. Activities were done to determine the following: 1) status of *Ralstonia solanacearum* in farmers' and experimental fields, 2) effects of control components including seed selection during storage, crop rotation, field sanitation, mulching, and manuring with beneficial organisms added, on *R. solanacearum* population development in fields and potato yield.

Soil, plant and tuber were taken from 83 locations in 13 villages in the Pangalengan District of West Java, Indonesia. Virulence of *R. solanacearum* population was determined using the Kiraly methods (1970), positive *R. solanacearum* reaction by NCM ELISA using Priou methods (1999), and Race and Biovar detection using the Hayward method (1964). Race 1 Biovar 3 and Race 3 Biovar 2 were identified. All the isolates from wilt-affected potato plants collected from the 83 locations were highly pathogenic to tomato and potato but were less virulent to eggplant, pepper, and ginger. Race 3 Biovar 2 was found at more than 1,300 m asl, while Race 1 Biovar 3 was found at 800 - 1500 m asl.

Seeds selected four times at one-month intervals in Diffuse Light Stores resulted in 100 percent of plants showing no wilt symptoms when subsequently planted. Yield per plant was higher compared with the farmers' practice. The rate of bacterial wilt disease development was lower following a 3-month maize rotation, a 3-month cabbage rotation, and a 3-month bean rotation. Apparently, *Pseudomonas fluorescens* applied one month after planting increased the avirulent population. Field sanitation, by using black silver plastic mulch to cover root and stem debris after harvest and to control weeds, improved yield production compared with that of the untreated plots (2 intervals of handweeding). The indigenous *Trichoderma* spp. and *Lactobacillus* spp. populations contributed to the development of healthy plants. Further research in collaboration with farmers is being planned to study the effects of components on the pathogenicity of the bacteria and host resistance.

### Introduction

Bacterial wilt (*Ralstonia solanacearum*) or brown rot is a serious disease of potato and is a major limiting factor of potato production in Indonesia. The extensive number of plants affected by the bacterium includes not only important hosts, but also a number of weeds. Gunawan et al. (1987, 1999) reported an extensive number of hosts from more than 50 families of weeds that were affected by the bacterium. The importance of indigenous vegetation has been clearly established when potato wilted in a field considered to have virgin soil, that was never previously planted with a solanaceous crops (Jackson and Gonzalez 1979, Martin 1979).

In many instances, the incidence of bacterial wilt has decreased after the first planting with a susceptible host. Probably the mechanics of removing the soil layer after first planting has played an important role in decreasing inoculum in soil. Studies conducted by Jackson and Gonzalez (1979) reported that two common weeds, *Melampodium perfoliatum* and *Bidens*

*pilosus*, had wilt symptoms. However, the bacterium was also isolated from plants showing no symptoms at all. The effect of crop rotation and weed control was associated with a declining incidence of rot and increasing yield. It was reported that more than 50 species of weeds associated with the potato plant were the principal reservoir of inoculum for the next planting season (Gunawan et al. 1999, 2000, 2001a). In such instance, bacterial wilt was effectively controlled by a combination of soil sanitation and use of healthy planting materials (Gunawan et al. 2001b).

Most of researchers working on bacterial wilt have agreed over the years that the two principal means of controlling the disease are rotation and use of disease-free seed. However, in order to establish a rotation program, it is imperative to know the host range. As early as 1939, Smith has worked on host range to control the disease by crop rotation (Martin 1979)

Elphinston and Aley (1992) reported that control of bacterial wilt of potato has been done in many countries. In Rwanda, one crop rotation with any of the five principal crops grown there greatly reduced wilt incidence, but longer rotations showed no advantage. Devaux et al. (1987 *cit* Elphinston and Aley 1992) also reported that when rotation was followed by planting a resistant potato variety, wilt incidence was further reduced. In Columbia, potato strain damage was greatest with continuous potato planting or rotation of potato and tree tomato (*Cyphomandra betacea*), which is also susceptible. Rotating maize for 8 – 10 months with either potato or tree tomato gave good control (Navarro and Granada 1978 *cit* Elphinston and Aley 1992). Research on crop rotation as a control measure after a cropping of heavily infected potato showed that the bacterium survived least with either onion or carrot as rotation crops.

French (1996) reported that rotation with maize and beans achieved good control in Honduras. Intercropping potato with beans resulted in less spread of *Ralstonia solanacearum* than with maize as intercrop. Potato rotated with maize reduced the population of the bacterial wilt pathogen in the soil. Gunadi et al. (1999) and Gunawan et al. (2001a) showed that a 3-month maize rotation, a 3-month bean rotation, and a 3-month cabbage rotation with application of *Pseudomonas fluorescens*, has reduced the bacterial wilt pathogen population and percentage of wilted potato plants. Soil sterilant Dazomet, applied one month before planting potato, has reduced the bacterial wilt population in the soil (Gunawan et al. 2001b).

Compared with 5- to 10-cm planting depth, planting shallower at 2 cm resulted in less wilt from soil infestation, but yield was less, with more tubers turning green. Seed-borne wilt or latent infection has long been recognized as the principal method of disseminating the potato strain. The movement of seed tubers from an infected field at warm location to cooler sites has repeatedly been reported to produce latently infected seeds in healthy-looking fields. Use of infected seeds has often resulted in serious outbreaks or even epidemics of bacterial wilt in the field. The use of clean seeds (pathogen-free) will reduce inoculum in the soil for next planting (Basuki et al. 1999).

Gunawan et al. (2000) reported that selection of potato seed in the storage before planting improved the quality of seeds. The use of Diffuse Light Storage (DLS) resulted in strong seeds and vigorous sprouts, compared with those in dark storage. Incidence of all pests and diseases were reduced when potato was stored in DLS at 30-day intervals. The conditions in DLS stimulated the sprouting hormone by sunlight (Asgar et al. 1994). DLS increased the quality of potato seeds by around 15 percent compared with those kept in the dark storage (Nainggolan 1993), but only a few seed growers have used the DLS technology (Gunawan et al., 2000).

The development of bacterial wilt associated with weed species was recorded in more than 50 weeds that have been growing well in potato fields in Pangalengan, Lembang and

Garut in Indonesia. They showed no symptoms that they were hosting *R. solanacearum* (Gunawan et al. 1999). Cortbaoui (1997) reported that the use of mulch technology in potato cultivation has many benefits: it controls weeds, reduces competition for nutrients and space, maintains relative humidity and soil structure, reduces soil and microelement erosion, increases soil fertility, and minimizes the damage caused by full sunlight and rain. Using mulch technology resulted in high yields and clean tubers compared with conventional weed control methods (Unger 1987 cit Reinjtjes et al. 1999). Moreover, using mulch in potato cultivation helped the roots to penetrate the soil easily, reduced weeds, and increased yield (Gunawan et al. 2000).

FAO (1984a cit Reinjtjes et al. 1999) reported that soil fertility caused by erosion has decreased in more than 554 million ha of lands. Soil productivity has been lost in the United States of America (30%), Africa (16.5%), West Asia (20%), and Central America (30%) due to erosion. Generally in the tropics, farmers planting potato in the highland and heterogeneous environments entails very high risks. Sustainable agriculture technology need to be used to maintain the natural resources productivity to increase environmental quality and economic sustainability (TAC, CGIAR 1988 cit Reinjtjes et al. 1999).

## **Materials and Methods**

Research was conducted on January to December 2001 to determine the following: status of *Ralstonia solanacearum* in potato cultivation, impacts of seed selection, plant rotations, field sanitation, weed control using black silver plastic (BSP) and using effective microorganism to as a strategy for increasing production and for the control of bacterial wilt in the soil.

Laboratory work on 83 soil samples was conducted at the Research Institute for Vegetable (RIV) in Lembang. The samples were collected at a depth of 40cm at regular intervals during the rotation periods in 83 location (in 13 villages) in the fields of Pangalengan, Bandung District, West Java Province, Indonesia (800 m - 1500 m asl). One gram of the soil from each sample was thoroughly mixed with 9 ml of sterilized distilled water (SDW) until 7 fold series. A 0.1 ml of suspension of each sample was purified on Tetrazolium (TZC) agar medium (Kelman 1954), incubated at 30°C for 72 hours until a fluidal colony was observed to be growing well. A virulent colony showed typical fluidal, smooth, white appearance with red internal whirling patterns while avirulent ones were dark red and round. Virulence was calculated and determined using the Kiraly method (1970). All colonies were tested using NCM ELISA. For pathogenecity tests, pure isolates were inoculated to tomato, potato, pepper, eggplant, and ginger in a greenhouse.

Isolates from all locations were tested for Biovar and Race groups using the Hayward method (1964). By using six carbohydrates, a positive reaction shown by a color change means that an antigen of the bacteria was present in the sample carbohydrate media for Biovar (Hayward 1964). Biovar of *R. solanacearum* was determined based on the use of six carbohydrates: cellobiose, lactose and maltose, oxidation of the hexosa alcohol, manitol and sorbitol. A 0.1 ml of *R. solanacearum* suspension was added to 3 ml of sterilized basal medium, consisting of ammonium dihydrogen phosphate (1.0 g), potassium chloride (0.2 g), magnesium sulphate (0.2 g), pepton (1.0 g), bromothymol blue (0.03 g), agar (3.0 g) and distilled water (1000 ml), and 5 ml each of the carbohydrates. The pH of the medium was 7.0 to 7.1 (an olivaceous green color). The mixed suspensions were left for 3, 7, and 14 days. The suspension changed color from olivaceous green to yellow, an indication of increasing acidity. The procedure for classifying of *R. solanacearum* is shown in Table 1.

Field experiments were conducted in the highlands (1,300 m asl) at farmers' fields in Cinangsi, Pangalengan. The four fields used had similar soil types (Andosol), and were infected with Race 3 Biovar 2. From January 2001 to December 2001, selected potato seeds in storage were studied regarding the storage's impact on the development of bacterial wilt and on yield. Paired comparisons were used and 10 were replicated. The improved (researchers') practice was compared with the farmers' practice.

To ensure uniform inoculum distribution in the soil, the blocks were located in areas of the field where the previous potato crop had been uniformly affected. Land was divided into two sites measuring 500 m<sup>2</sup> each. Each was surrounded by drainage ditches 30 cm wide and deep between blocks, and 50 cm wide and deep between treatments. Seeds were planted in double rows in each block of treatments. Black Silver Plastic (BSP) for weed control was used.

To determine time frequency and kinds of plants for rotation, three-months each of the following crops were rotated: maize, cabbage, and beans. Potato was planted for three months before rotation to develop bacterial wilt inoculum. A randomized block design was used. For each treatment, an effective microorganism (EM), *P. flourescens*, was added. Field sanitation and weed control by mulching were conducted in the farmers' fields with two treatments: with and without BSP. Paired comparisons were done in two blocks of 500 m<sup>2</sup> each.

Effect of the effective microorganisms or EM (*Trichoderma* spp. and *Lactobacillus* spp.) on the quality of manure was also determined in the farmers' fields. The manures used were those of sheep, chicken, cow, horse, and paddy straw compost. EM-treated and untreated manure were applied on the fields and the plants were observed for disease incidence, population of BW, weed species, vegetative growth, and generative growth. Results were recorded on weekly and monthly intervals.

## **Results and Discussion**

The rate of disease development of the virulent population was higher (13.9 x 10<sup>7</sup> cfu/g - 45.0 x 10<sup>7</sup> cfu/g soil) than the avirulent population (0.9 x 10<sup>7</sup> cfu - 7.5 x 10<sup>7</sup> cfu/g.soil) as shown in table 2.

Informal surveys were conducted to determine farmer behavior on potato cultivation. Results showed that farmers have never consciously evaluate the kinds of vegetables they select for rotation after planting potato. A few farmers intercropped tomato or pepper with potato. One farmer reasoned that these vegetables were profitable. Indirectly, farmers have played a role in sustaining the bacterial wilt inoculum in their fields. Another probable reason for the high bacterial wilt population is the nutritional resources abundant in nature.

The farmers have never sanitized their fields of infected stems or infected tubers after harvest. All debris in the area were left until the next planting season. Moreover, weeds in areas planted to potato have promoted the survival of the bacterium by serving as inoculum hosts. These weeds were symptomless (Abidin and Gunawan 2000).

It has been proven that land sanitation and weed control reduce bacterial population in soil (Gunawan et al. 2001b). The use of the soil sterilant Dazomet reduced bacterial wilt in the soil.

## **Biovar classifications of *Ralstonia solanacearum***

Bacterial wilt can be determined by the bacteria's utilization of the disaccharides cellobiose, lactose and maltose, and the oxidation of the hexose alcohols dulcitol, mannitol, and sorbitol. All 83 isolates oxidized all the carbohydrates. The Hayward method classified *R. solanacearum* isolates from the soil as Race 1 Biovar 3 and those from infected stems and tubers as Race 3 Biovar 2. Smith *cit* Kelman (1954) reported that Race 3 Biovar 2 was only found in an infected potato plant and tuber. Gunawan et al. (1999) reported that Race 1 Biovar 3 was detected only in the rhizosphere of weeds and in the soil. All collected bacterial wilt were positive in NCM ELISA (table 3).

## **Pathogenicity tests**

*R. solanacearum* was found to be pathogenic to: tomato and potato but not to pepper, eggplant, and ginger (table 4).

## **Impacts of seed selection**

The potato fields showed healthy-looking plants with no pest and disease incidence. There were no symptoms caused by *R. solanacearum*. After 90 days, plants that were seed selected by the improved way were taller, nearly 115-cm tall with a canopy diameter around 46-49 cm (table 5). Those produced by the farmers' practice averaged 113 cm in height with canopy diameters of 43-47 cm. Seed selection is the main factor in increasing plant health and yield.

## **Pests and diseases of potato**

Generally, all the plants looked healthy in both treatments, with no severe pest and disease damage 90 days after planting. Apparently, good selection of seeds resulted in healthy plants with no latent seed infection and with strong and big stems (more than 1 cm diameter). Late blight observed at 60-70 days after planting was controlled by systemic fungicide sprayed at 10-day intervals. Early late blight symptoms were treated mechanically (cut with disinfected scissors dipped in soap solution). *Thrips palmi*, *Pthorimaea operculella*, and *Myzus persicae* were controlled using an integrated pest management (PHT-SDT/SDR Rintisan 1993) with an insecticide recommended by Balitsa (RIV). Cooler weather has reduced these pests' population.

Fungi wilt caused by *Fusarium oxysporum* was noted in fields managed by farmers' conventional practice and was eradicated. Probably, a farmer found it difficult to isolate dry rot when it became associated with an earlier infection by potato tuber moth (PTM) in the same place on the surface of seed potato.

## **Generative growth of potato**

Yield production from both treatments (improved and farmers' practices) resulted in tuber size variations. Tuber sizes classified into six grades according to weight: Super A1 = more than 250 g/tuber, A1 = 200-250 g/tuber, A = 150-200 g/tuber, B = 60-150 g/tuber, C = 50-60 g/tuber, and D for less than 40 g/tuber. Data on the distribution of yield by tuber size are shown in Tables 6.

Yield from sample tubers (10 plants) showed several disease symptoms caused by *R. solanacearum*, *Erwinia carotovora*, *Fusarium oxysporum*, and *Grylotalpa* spp (table 7). Potato tuber moth (*P. operculella*) was found to have damaged at most two tubers from the total sample (table 8).

### **Population of *R. solanacearum* in the soil**

The population of *R. solanacearum* in the soil was recorded before and after planting with potato (table 9). Initial population of the virulent group before planting was  $0.30 \times 10^7$  cfu/g soil, while that of the avirulent group was  $2.70 \times 10^7$  cfu/g soil with the avirulent group. The population of the virulent group increased 90 days after planting of potato. Perhaps, exudates from plant metabolism nourished the pathogen, and favored bacterial wilt survival and development in the rhizosphere of potato (Johnson and Curl 1972).

### **Effects of time frequency and kinds of rotation plants**

In a three-month maize rotation applied with *Pseudomonas fluorescens* a month after planting potato, the virulent population had a lower rate of disease development than the avirulent population (table 10). The root system of maize releases toxins for bacterial wilt..

The bacterium's population decreased following three months each of bean and cabbage rotations (table 11). The 3-month bean rotation and 3-month cabbage rotation, applied with the antagonistic microorganism *P. fluorescens*, reduced the population of *R. solanacearum* at 30, 60, and 90 days after planting. It is probable that the population of beneficial microorganisms was supported nutritionally the rotation crop, as reported by Johnson and Curl (1972).

### **Field sanitation and weed control by mulching**

Weeds collected in the plots were: *Drimaria cordata*, *Galinsoga perfoliatum*, *Bidens pilosus*, *Ageratum conyzoides*, *Amaranthus spinosus*, *Oxalis* sp, *Polygonum* sp, *Datura stramonium*, *Cyperus* spp, and grasses.

Generally, the potato vegetative growth looked good in the field. On average, the plants were 115-cm tall with 51.8-cm canopy diameter in the field that used black silver plastic mulch. At the hand-weeded fields, the plants' average height was 100 cm, while canopy diameter averaged 40.5 cm. The short plants and small canopy diameter in the untreated field probably resulted from competition with the weeds for nutrients and space.

The use of mulch suppressed weed growth and development, eliminated space competition, and allowed the soil nutrients to be solely taken up by the crops. Abidin et al. (2000) observed that weeds used more nutrients than the main crop, resulting in low yields for the crop. The use of mulch maintains soil relative humidity and helps roots penetrate the soil, among other things (Cortbaoui 1997). Differences of yield between plots treated and untreated with mulch are shown in table 12.

Organic manures decomposed with *Trichoderma* spp and *Lactobacillus* spp included chicken manure, sheep manure, cow manure, horse manure, and paddy straw compost. Height of plants applied with the EM-decomposed organic manure varied from 95-102 cm while canopy diameters varied from 40-45 cm. The untreated plants had heights and canopy diameters of 75-80 cm and was 40-43 cm, respectively, 90 days after planting. All plants looked healthy, and plants treated with manure showed more vegetative growth (table 13). Yield of

tubers from plants treated with organic manures was higher than from untreated plants (table 14). Probably the organic manure decomposed with EM has improving the nutrients intake by plants and has utilized for it photosynthetic.

## **Conclusions**

1. *Ralstonia solanacearum* collected from the soil and weeds belonged to Race 1 Biovar 3 and those collected from infected tubers and stems of potato belonged to Race 3 Biovar 2. All the samples showed high virulence.
2. Pathogenicity tests showed that all the bacterial wilt isolates were virulent to tomato and potato and less virulent to pepper, ginger and eggplant. All isolates were positive using NCM ELISA.
3. Seeds selected four times in a monthly interval in the store, and once before planting, resulted in plants with strong and healthy vegetative growth with no latent seed infection.
4. Sprouts of seeds, when placed in Diffuse Light Store, showed improved quality.
5. Black silver plastic (BSP) mulch reduced the development of weeds, pests, and disease.
6. Virulent *R. solanacearum* population declined following a 3-month maize rotation. Avirulence was increased in the rhizosphere of maize.
7. The rate of disease development was lower following a 3-month rotation each of maize, bean, and cabbage, that was applied with *Pseudomonas fluorescens*. Soil management, land sanitation, and weed control with BSP mulch were also employed. Compared with handweeding, the use of BSP increased plant health and produced clean tubers. The plastic mulch suppressed weed growth, smoothed soil structure, made potato harvesting easier, showed cleaner potato yield, maintained relative humidity, and prevented soil erosion.
8. The IPM method lowered the intensity of pests and diseases.
9. 9. Using manure decomposed with *Trichoderma* spp and *Lactobacillus* spp resulted in healthy plants.

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**Table 1. Procedure for classification of *Ralstonia solanacearum***

Physiological Tests	Biovars				
	1	2	3	4	5
Utilization of disaccharides:					
Cellobiose	-	+	+	-	+
Lactose	-	+	+	-	+
Maltose	-	+	+	-	+
Oxidation of alcohols:					
Dulcitol	-	-	+	+	-
Mannitol	-	-	+	+	+
Sorbitol	-	-	+	+	-

Notes: Bv determination is based on the following: Bv1= utilization and oxidation tests negative; Bv 2 = utilization disaccharides, does not oxidize the alcohol; Bv 3 = utilization and oxidation both positive; Bv 4 = utilization of disaccharides negative, oxidizes the hexosa alcohol; Bv5 = utilization disaccharides, oxidizes mannitol but not dulcitol or sorbitol

**Table 2. Virulence characteristics of *Ralstonia solanacearum* in the soil of potato fields, Pangalengan, West Java**

Code No	Desas	Location	Elevation(m) above sea Level	<i>R. solanacearum</i> ( $10^7$ cfu)/g.soil sample	
				Virulent	Avirulent
1	1Pangalengan	Bbk. Laksana	1310	21.8	2.1
2		Legok Kandang	1300	28.9	1.9
3		Jublegan	1310	15.4	1.1
4		Ciawi koral	1300	30.0	2.1
5		Pasanggrahan	1300	13.9*	2.3
6		Bojong	1300	24.8	1.8
7		Pangalengan	1300	15.6	1.0
8.	2Marga Mulya	Pada Awas	1300	25.6	1.1
9		Legok Bako	1280	16.9	2.0
10		Norogtog	1290	21.3	1.9
11		Kebon Kopi	1290	21.0	1.2
12		Marga mulya II	1320	32.5	1.4
13	3.Marga Mukti	Sukamenak Kln	1380	17.5	3.5
14		Sukamenak Wtn	1380	32.5	2.7
15		Cibeurum	1370	30.5	1.2
16		Cipanas	1390	35.2	1.2
17		Ranca manyar	1390	20.2	2.2
18		Pangkalan	1390	18.6	1.9
19		Kerta manah	1400	26.5	1.7
20		Cikole	1400	20.0	1.3
21		Los Cimaung	1450	31.2	1.6
22	4.Marga Mekar	Suka Mulya	1400	34.2	0.9*
23		Bbk Kiara	1450	30.5	2.4
24		Cieurih	1390	28.2	1.4
25		Los kulalet	1390	22.1	1.8

26		Kramat	1390	23.3	1.3
27		Cisangkuy	1300	22.0	3.9
28	5.Sukamanah	Sukallah	1480	14.2	4.1
29		Los	1500	18.7	3.2
30		BBI	1500	21.5	1.7
31		Kertamanah	1500	23.5	2.1
32		Citere	1470	19.5	1.4
33		Pintu	1500	17.6	1.4
34	6.Wanasuka	Cipanas	1500	35.7	1.3
35		Srikandi	1490	28.5	1.2
36		Pasir yunghun	1500	23.2	1.0
37		Kiara roay	1500	19.6	0.9
38		Wanasuka	1450	30.6	1.8
39	7.Banjar sari	Malabar	1510	35.7	1.4
40		Suka ratu	1510	40.0	1.7
41		Babakan	1500	28.6	2.0
42		Cibolang	1510	22.9	2.2
43		Banjar sari	1500	37.2	3.1
44	8.Tribakti Mulya	Baru petak	1050	37.3	7.5*
45		Ranca gada	1100	40.4	4.2
46		Patrol	1100	44.3	3.0
47		Cirancah	1100	29.0	1.6
48		Cihideung	1100	36.8	1.6
49		Lebak saat	1120	33.4	1.2
50	9.Lamajang	Ciburuy	910	29.5	4.5
51		Cibiana	900	45.0*	3.9
52		Badra	850	44.1	2.1
53		Karang tengah	800	43.3	2.1
54		Babakan tipah	850	39.0	1.4
55		Panenjoan	850	41.0	1.7
56	10.Pulosari	Taraju	1350	23.6	4.2
58		Sirnasari	1360	42.2	3.3
59		Dangdang	1360	28,0	1.2
60		Kiaragede	1360	30.0	2,8
61		Cinangsi	1350	21.5	4.8
62		Kiarasanding	1360	37.0	5.2
63	11.Sukaluyu	Ciseke	1400	34.5	5.4
64		Wates	1400	33.0	6.5
65		Satani	1420	42.1	6.0
66		Cilaki	1410	31.0	4,6
67		Dalima	1450	38.4	2.2
68		Busmir	1400	29.4	2.7
69	12.Margaluyu	Baruipukan	1480	37.8	3.3
70		Barukampak	1480	42.3	5.4
71		Puncakraya	1420	44.2	6.5
72		Puncangmara	1420	25.8	6.2
73		Baruwangi	1490	28.2	5.5
74		Cikole	1480	33.0	7.2
75		Gunungcupu	1490	34.2	3.2
76	13.Warna sari	Padahurip	1380	34.2	1.2

77		Warnasari tengah	1360	27.3	3.2
78		Kiaracondong	1350	41.0	2.4
79		Citus	1350	34.2	2.5
80		Cibeting	1370	22.7	3.4
81		Palayangan	1380	21.7	5.1
82		Cipangisikan	1370	32.5	2.1
83		Parabon	1370	33.0	2.3

Notes :Cfu colony form unit. G= gram, R= *Ralstonia* The bacteria population is resulted and was recorded from averaged of two petri dish.

**Table 3. *Ralstonia solanacearum* isolates collected and oxidized to six carbohydrates and NCM ELISA**

Desas was surveyed	Race and Biovar from the soil		Race and Biovar in potato tubers and stems		NCM ELISA
	Race	Biovar	Race	Biovar	
1.Desas Pangalengan	1	3	3	2	+
2.Desas Margamulya	1	3	3	2	+
3.Desas Margamukti	1	3	3	2	+
4.Desas Marga mekar	1	3	3	2	+
5.Desas Sukamanah	1	3	3	2	+
6.Desas Warna sari	1	3	3	2	+
7.Desas Banjarsari	1	3	3	2	+
8.Desas Tribaktimulya	1	3	3	2	+
9.Desas Lamajang	1	3	3	2	+
10Desas Pulosari	1	3	3	2	+
11Desas Sukaluyu	1	3	3	2	+
12Desas Margaluyu	1	3	3	2	+
13Desas Warnasuka	1	3	3	2	+

Note + = positive reaction in utilization disaccharides or oxidation of *Ralstonia solanacearum*, negatif = does not occur oxidize the alcohols and utilizatin of disaccharides.

**Table 4. Pathogenisity test of *R. solanacearum* isolates from the soil after planting**

Desas was surveyed	<i>R.solanacearum</i> suspension( $10^7$ cfu/ml)							
	Tomato	Pepper		Eggplant		Potato	Ginger	
1.Desas Pangalengan	+	+	-	+	-	+	+	-
2.Desas Margamulya	+	+	-	+	-	+	+	-
3.Desas Margamukti	+	+	-	+	-	+	+	-
4.Desas Marga mekar	+	+	-	+	-	+	+	-
5.Desas Sukamanah	+	+	-	+	-	+	+	-
6.Desas Warna sari	+	+	-	+	-	+	+	-
7.Desas Banjarsari	+	+	-	+	-	+	+	-
8.Desas TRibaktimulya	+	+	-	+	-	+	+	-
9.Desas Lamajang	+	+	-	+	-	+	+	-
10Desas Pulosari	+	+	-	+	-	+	+	-
11Desas Sukaluyu	+	+	-	+	-	+	+	-
12Desas Margaluyu	+	+	-	+	-	+	+	-
13Desas Warnasuka	+	+	-	+	-	+	+	-

Notes: + = positive reaction ( pathogen), - + non pathogen or symptomless

**Table 5. Vegetative growth of potato crops resulting from seed selection**

Treatment	Seed selection using researcher practice	Seed selection using farmers' practices
	Cm	Cm
Height of plant 30 dap	25.0	21.0
Height of plant 60 dap	80.0	75.0
Height of plant 90 dap	115.0	113.0
Diameter of canopy 30 dap	35.0	35.0
Diameter of canopy 60 dap	46.0	43.0
Diameter of canopy 90 dap	49.0	47.0
Diameter of stem 30 dap	1.0	1.0
Diameter of stem 60 dap	1.5	1.3
Diameter of stem 90 dap	2.1	2.0

Note :dap = day after planting.

**Table 6. Tuber yield from plant samples resulting from seed selection practices**

Treatment	Yield/kg/ plant	Total no. of tubers	Grade of tuber (% of total yield)				
			AL	L	A	B	C
Seed selection using researcher practice	1.72	23.9	2.3	1.15	17	40	39
Seed selection using farmers' practices	1.53	23.5	1.7	1.0	11	33	56

Notes:AL= more than 200g/tuber, A=: more than 150g/tuber, B=: more than 60 -100g./tuber, C:= more than 50-60 g

**Table 7. Diseases infecting tubers following seed selection practices**

Treatment	Percentage of infected tuber caused by			
	Dry rot	Nematode	Soft rot	Brown rot
Seed selection using researcher practice	0.1	0.0	1.5	0.1
Seed selection using farmers' practices	0.1	0.0	2.1	0.2

**Table 8. Pests infecting tubers following seed selection practices**

Treatment	Percentage of infected tuber caused by	
	<i>Ptheromaea. Operculella</i>	<i>Gryllotalpa spp</i>
Seed selection using researcher practice	0.0	0.3
Seed selection using farmers' practices	0.0	0.4

**Table 9. Population of *Ralstonia solanacearum* in soil before and after planting with potato**

Treatment	<i>Ralstonia solanacearum</i> population (10 <sup>7</sup> cfu) in the soil							
	Before planting.		30 DAP		60 DAP		90DAP	
	.V	AV	V	AV	V	AV	V	AV
Seed selection using researcher practice	0.30	2.70	1.30	3.80	1.70	3.50	2.20	3.90
Seed selection using farmers' practices	0.30	2.70	1.40	2.50	1.80	3.75	2.50	3.60

Note: cfu = colony form unit; dap = day after planting. V= virulent, AV= avirulent.

**Table 10. Population of *Ralstonia solanacearum* in the soil planted with maize**

Treatment	Population of <i>Ralstonia solanacearum</i> (10 <sup>7</sup> cfu) in the soil							
	After potato		30 dap with maize		60dap with maize		90dap with maize	
	Virulent	Avirulent	Virulent	Avirulent	Virulent	Avirulent	Virulent	Avirulent
Maize+ Pf	1.20	2.60	0.05	7.75	0.02	1.75	0.01	9.50
Maize+ Pf	1.56	2.30	0.04	9.50	0.02	8.00	0.00	10.00
Maize+ Pf	1.90	1.70	0.02	9.50	0.01	7.55	0.01	10.50
Maize+ Pf	1.10	3.50	0.02	5.50	0.01	7.75	0.04	7.75
Maize+ Pf	1.35	3.50	0.03	10.50	0.04	9.50	0.03	10.50
Maize+ Pf	2.00	2.90	0.04	10.50	0.03	8.65	0.03	10.50

**Table 11. Population of *Ralstonia solanacearum* in the soil planted with beans and cabbage as rotation crops**

Treatment	Pop.of Vir and Avir BW after maize		Virulent population of BW(10 <sup>7</sup> cfu)/g soil			Avirulent population of BW (10 <sup>7</sup> cfu)/g soil		
	90dap	90dap	30dap	60dap	90dap	30dap	60dap	90dap
Potato	0.10	9.50	0.01	1.50	2.00	10.70	12.00	11.50
Cabbage	0.00	10.00	0.02	0.05	1.50	22.30	22.50	21.50
Beans	0.10	10.50	0.02	0.04	0.04	20.20	28.00	37.50
Potato+ Pf	0.04	7.80	0.03	0.04	0.03	10.50	21.50	20.20
Cabbage+ Pf	0.03	10.50	0.03	0.04	0.04	20.30	22.00	21.50
Beans + Pf	0.04	10.50	0.03	0.03	0.04	20.20	40.40	55.20

Note: Vir= virulent, Avir+ avirulent, CFU= colony form unit., g= gram, BW= bacterial wilt. Population of Bacterial wilt recorded from the average from two petri dish

**Table 12. Effect of black silver plastic mulch on yield and pest and diseases on tuber**

Treatment	Tuber yield/plant and percentage of infected tubers						
	Healthy yield/kg	Percentage of infected tuber					
		<i>R.sol.</i>	<i>E.car.</i>	<i>F.oxy.</i>	<i>P.oper.</i>	<i>Gryl.</i>	Nem.
BSP mulch	1.70	0.30	0.5	0.4	0.2	0.2	0.2
Without BSP mulch	1.00	0.20	0.7	0.3	0.5	0.1	0.2

Note: *R.sol* = *Ralstonia solanacearum*, *E.car* = *Erwinia carotovora*; *F.oxy*= *Fusarium oxysporum*; *P.oper*= *Ptheriomaea operculella*; *Gryl*= *Gryllotalpa*; Nem= nematoda.

**Table 13. Vegetative growth of potato plants after using organic manures decomposed with effective microorganisms (EM)**

Treatment	Plant height (cm) and canopy (cm)					
	30 dap	60 dap	90dap	30 dap	60 dap	90 dap
<i>Trichoderma spp</i> applied to:						
Chicken	25.5	60.5	105.0	21.2	35.0	42.0
Sheep	25.0	64.0	107.0	22.2	34.0	45.0
Cow	25.0	65.0	98.5	21.2	32.4	43.0
Horse	25.0	63.0	95.0	21.4	30.2	44.5
Paddy straw compost	26.0	65.0	110.0	21.4	33.5	44.5
<i>Lactobacillus spp</i> applied to:						
Chicken	25.0	62.5	101.0	22.2	30.2	40.2
Sheep	25.0	60.2	100.5	23.0	33.2	42.0
Cow	26.0	65.0	91.0	22.6	32.0	40.2
Horse	25.5	60.0	90.0	22.4	30.5	40.2
Paddy straw compost	25.5	60.0	105.0	22.5	30.5	40.0
Untreated						
Chicken	23.5	55.5	85.2	20.0	30.0	35.0
Sheep	25.0	53.4	83.2	21.0	29.0	39.0
Cow	25.0	52.0	85.0	20.5	29.0	39.0
Horse	25.0	49.7	80.0	22.0	30.0	36.0
Paddy straw compost	25.0	50.0	83.3	20.2	30.0	35.5

**Table 14. Potato yield comparisons from manure treatments**

Treatment	Potato yield using different manures (kg/plant)				
	Chicken manure	Sheep manure	Cow manure	Horse manure	Paddy straw Compost
<i>Trichoderma spp</i>	0.80 -1.10	0.80-1.10	0.90- 0.95	0.75-1.00	1.00- 1.10
<i>Lactobacillus spp</i>	0.90 -1.00	0,95-.0.95	0.80-.0.90	0.75-0..80	0.90- 1.00
Untreated	0,80-1.00	0.70-1.00	0.80 -0.85	0.75-0.80	0.90-.0.95