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## Effects of compost amendments on the suppressiveness of *Ralstonia solanacearum* (Smith) Yabuuchi on potatoes in western Highland of Cameroon

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### Abstract

The study was conducted to evaluate the effects of compost on bacterial wilt of potatoes. Six compost amendments were built using 3 grass species (*Ageratum haustonianum*, *Pennisetum purpureum*, and *Tithonia diversifolia*) and 2 animal dung (cow dung and poultry manure). The trial had 7 compost types (treatments): 6 treatments with different composts at 50% and sterilized soil (control). The experimental layout was a complete randomized design with 10 replicates. Potato plants were inoculated 30 days after planting with 30 ml of bacterial suspension at  $10^9$  cfu/ml per plant and evaluated weekly for wilt incidence and severity. Fluorescent bacterial populations of compost were quantified 24 h after plating on MacCkonky agar. Physicochemical properties of soil and composts were analyzed. Bacterial wilt incidence and severity were significantly reduced with the incorporation of compost irrespective of the compost type. The most suppressive compost to bacterial wilt was *T. diversifolia* and poultry manure (disease incidence: 7.5% and disease severity: 1.3%), with the highest population of fluorescent bacteria ( $20 \times 10^{11}$  cfu/ml). The study suggests that *T. diversifolia* and poultry manure compost could be incorporated to an integrated control strategy of bacterial wilt on potato. The antagonistic bacteria in *T. diversifolia* + poultry manure compost will be studied later

**Keywords:** Bacterial wilt, disease control, *Solanum tuberosum*, Suppressiveness composts

### 1. Introduction

In all potato growing regions bacterial wilt caused by *Ralstonia solanacearum* (Smith) Yabuuchi is regarded as an important disease contributing to yield reduction [1]. It is one of the most damaging pathogens of potatoes [2] and can persist in the soil for considerable periods of time. This pathogen affects more than 200 plant species in over 50 families throughout the world [2, 3, 4]. The disease is most severe at temperatures of 24 – 35°C [5]. It has been estimated to affect about 3.75 million acres in approximately 80 countries throughout the world. Control of bacterial wilt is difficult due to its wide host range and presence of many pathogenic strains in Cameroon [6, 7]. Global economic losses due to the disease on potatoes have been estimated at US\$950 per year (Elphinstone *et al.*, 2005). The use of crop rotation is not feasible due to small farm sizes which hinder effective rotation programs [7]. Controlling this disease by resistant cultivars has been very ineffective since resistance in the host plant may vary with location and temperature; moreover, antibiotics have shown little efficiency in suppressing *R. solanacearum* in the field [2]. The use of soil amendments with organic matter has been adopted for controlling certain soil borne diseases including bacterial wilt [8]. Studies have shown that one way of stimulating bio control in the soil is the use of organic matter [9]. Efficient soil management generally improves the composition and activities of microorganisms, thereby enhancing the biological control capacity of the soil [9]. So far, information on the effect of organic matter on the suppressiveness of *R. solanacearum* is still limited. Hence, the objective of this study was to increase potato production through the control of bacterial wilt by using compost.

### Materials and method

Study area, potato variety and preparation of compost

The trial was conducted in the screen house of the Plant Pathology laboratory of the Faculty of Agronomy and Agricultural Sciences (FASA), University of Dschang. Certified potato seed tubers (variety CIPIRA) were used. The average weight of the tubers (determined from 10 randomly selected tubers) was 24 g. Plants used for composting, animal dung (cow dung and poultry manure) were collected from the application and research farm (ARF) of FASA. Plants were composed of goat weeds (*Ageratum houstonianum* L.), elephant grass (*Pennisetum purpureum* Schumacher) and sunflower (*Tithonia diversifolia* Hemsl.). Composting of plant and

animal dung was conducted according to the following combinations: Compost with goat weed + cow dung, Compost with goat weed + poultry manure, Compost with elephant grass + cow dung, Compost with elephant grass + poultry manure, Compost with Sunflower + cow dung, Compost with Sunflower + poultry manure. Plant species were chopped into pieces of about 10 cm, to facilitate decomposition. The layers of grass were 10 cm thick for elephant grass, 13 cm for goat weed and 15 cm for sunflower. Animal dung was 2 cm thick and served as a source of microorganisms necessary for grass decomposition process. Each compost heap had a chimney for ventilation, thereby facilitating microbial activities. The compost was turned every fortnight so as to facilitate a homogenous decomposition, improve aeration and encourage the composting process. Each compost was covered with plantain leaves under dry weather so as to prevent water loss by evaporation. Monitoring had to do with regular observation of the compost for changes in temperature, odor and color. At maturity, the temperature remained constant at 28°C.

#### **Soil sterilization and determination of physicochemical properties of soil and compost**

Soil samples from the ARF of FASA were sterilized twice in an autoclave at a temperature of 121°C and a pressure of 1 bar for 20 min [10]. After sterilization, potato tubers were sowing in five pots containing sterilized soil. These potato tubers grow without developing wilt symptoms. This indicates that no bacterium (*R. solanacearum*) were present in the sterilized soil. The soil and compost samples were analyzed in the Soil Analysis and Environmental Chemistry laboratory of the University of Dschang for the pH, exchangeable bases, organic matter content, nitrogen content and base saturation. Samples were air dried before grinding and sieving to obtain fine particles (< 2 mm).

#### **Isolation of the pathogen and pathogenicity**

The pathogen (*R. solanacearum*) was isolated from an infected potato plant collected from the ARF of FASA. An infected stem was washed with sterile distilled water and surface sterilized by wiping the cut sections with a tissue soaked in 70% alcohol. Pieces of 2 - 3 cm long were cut from it and transferred into 10 ml of sterile distilled water in a test tube [13]. This setup was allowed to stand for 24 h until the bacterial ooze turned the water into a milky color. Drops of the bacteria solution were incubated on a potato sugar agar (PSA) medium in Petri dishes for 24 h at 30°C [13, 14]. At the end of the incubation, the plates were examined for single colonies of bacteria, which were multiplied in sterilized distilled water for 24 h. A Gram reaction test was carried out by stirring bacteria colonies for 24 h in a few drops of 3% KOH on a slide for 5 – 10 s and pulling up with a loop [15]. Then, five potted potato plants were inoculated with 30 ml bacterial solution for a pathogenicity test.

#### **Preparation of the bacterial solution for inoculation**

A bacterial solution of 500 ml was prepared and allowed to multiply at 30°C for 48 h. A ten-fold serial dilution was made from it and 0.1 ml from the 7<sup>th</sup> to the 10<sup>th</sup> dilutions were plated on PSA and incubated for 24 h [14]. The number of living bacteria (or colony forming units, CFU) on the Petri dishes were used to determine the concentration of bacterial colonies in the stock solution according to following formula [12]  $CFU = \text{Number of colonies per ml plated} / \text{total dilution factor}$ .

#### **Identification of microbial antagonists in the compost**

The main microbial antagonists were identified both in the compost material and soil so as to determine the materials that contained biological active microbes. Fluorescent bacteria were determined using MacConkey agar. The dilution plate method was used to determine the microbial population density in the compost types [12]. The process was as follows: samples of the composts were air-dried, grind lightly and thoroughly mixed. Two saline tablets were dissolved in 1000 ml of hot water at 70°C and sterilized at 121°C for 15 min. A 10 g subsample of each compost type was transferred to a 90 ml flask containing the NaCl solution. From this solution, a tenfold serial dilution was made using a pipette; then, 0.1 ml of the 10<sup>th</sup> dilution was plated in MacConkey agar in a Petri dish and incubated at 26°C for 4 days [16]. Colonies that develop were counted and the mean of each type was used to calculate the population density per compost type using the relation: number of bacteria cfu = number of colonies x 10<sup>n</sup>.

#### **Evaluation of the biological activity of composts**

Each compost type was mixed with sterilized soil in the ratio of 1:1. Ten pots were filed per compost type. Each pot had 2 kg of substrate. The control had 100% sterilized soil. A total of 70 pots were filed. One pre germinated potato tuber was planted in the center of each polyethylene bag at 10 cm deep. The experimental design was a completely randomized plan with 7 treatments and 10 replicates. The pots containing compost were inoculated with 30 ml of inoculums (10<sup>9</sup>cfu/ml) one month after planting while pots containing sterilized soil (control) received 30 ml of distilled water.

#### **Disease assessment**

Disease incidence and severity express in percent were assessed weekly. Disease incidence was the number of wilted plants over the total number of plants considered times 100, while disease severity was determined using a 1 to 5 rating scale in which 1 was for plants which did not show symptoms, 2 for plants with one leaf at least partially wilted, 3 for plants with 2 or 3 leaves wilted, 4 for plants with 4 or more leaves wilted and 5 for dead plants [17].

#### **Data analysis**

Data were analyzed by applying Analysis of Variance technique. A Student-Newman-Keuls (SNK) tests was used to compare means at probability threshold of 5% using the software SPSS. Microbial population density and physicochemical properties were regressed on disease severity.

#### **Results**

##### **Isolation and pathogenicity**

When fragments were cut from plant samples in which symptoms of disease were evident, fluidal pinkish red centered colonies, typical of *R. solanacearum* were observed on PSA medium. Typical isolated colonies were picked and purified for confirmation of bacterial wilt causing pathogen *R. solanacearum*. All the five potted potato plants inoculated with 100 ml bacterial solution develop the disease after 1 week.

##### **Physicochemical properties of compost**

The physicochemical analysis of soil and compost mixtures showed that compost mixtures with poultry manure recorded higher values of Ca, Mg and N contents than the ones recorded in compost mixtures with cow dung (Table 1).

**Table 1:** Physicochemical properties of compost and soil used in the study

Compost	K(meq/100g)	Na(meq/100g)	Ca(meq/100g)	Mg(meq/100g)	OM (%)	C/N	pH (H <sub>2</sub> O)	N (%)
Soil	0.03	0.10	1.32	2.20	7.42	10.6	6.75	0.407
Goat weed+ Poultry manure	0.25	0.10	10.50	37.50	15.48	7.1	7.45	0.833
Elephant grass + Poultry manure	0.33	0.34	33.50	57.10	14.64	8.7	7.40	0.973
Sunflower+ Poultry manure	0.33	0.10	44.70	69.90	15.39	5.5	7.47	1.610
Goat weed + Cow dung	0.33	0.10	32.30	20.30	15.57	10.8	7.36	1.267
Elephant grass + Cow dung	0.25	0.10	23.30	16.24	12.70	6.8	7.31	1.078
Sunflower + Cow dung	0.33	0.10	29.70	20.30	13.35	5.5	7.70	0.728

Soil samples had the lowest values of the constituents that were analyzed than the ones from the other compost mixtures. Soil had a pH of 6.75, compared with pH values of 7.31 to 7.70 for the compost types. Compost mixtures made with sunflower + poultry manure had the highest values for N (1.610 %), Ca (44.90 meq/100g) and Mg (69.90 meq/100g). The lowest values were recorded in soil samples (0.407 % N, 1.3 meq/100g Ca and 2.22 meq/100g Mg).

### Population of fluorescent bacteria

Fluorescent bacterial population showed that compost made with sunflower + poultry manure had the highest population density of  $20 \times 10^{11}$  cfu/ml while sterilized soil samples had none (Table 2).

**Table 2:** Microbial population density on the different compost types

Compost	Mean ( $\times 10^{11}$ cfu/ml)	N
Sunflower + Poultry manure	20.0 a *	3
Elephant grass + Poultry manure	9.8 b	3
Elephant grass + Cow dung	8.4 b	3
Goat weed + Poultry manure	3.03 c	3
Goat weed + Cow dung	3.03 c	3
Sunflower + Cow dung	1.83 c	3
Sterilized soil	0.00 c	3

\*Means within a column followed by the same letter are not significantly different according to SNK test at 5%. N = number of repetitions per compost type.

### Influence of compost amendments on bacterial wilt incidence and severity

The first expression of bacterial wilt was observed 14 days after inoculation on sterilized soil, and on compost made with sunflower + cow dung 20 days after planting. Disease incidence and disease severity were lowest in the compost made with sunflower and poultry manure; the highest disease incidence and severity was recorded on plants grown on sterilized soil (Table 3). There were no significant differences ( $p = 0.05$ ) of disease incidence and severity between the other compost types and the control. The situation of wilt incidence was correlated to that of severity. Results showed that potatoes grown on compost made with elephant grass and poultry had the least incidence, while the ones grown on sterilized soil had the highest. Plants grown on compost made with sunflower and cow dung had the highest incidence from the six compost types. However, plants grown on sterilized soil still recorded the highest severities (Table 3).

**Table 3:** Effects of compost types on bacterial wilt incidence and severity (%) on 8 weeks old planting materials

Compost	Disease incidence	Disease severity
Sterilized soil	63 a*	3.7 a
Sunflower + Cow dung	23.2 b	2.1 a
Goat weed + Cow dung	13.9 b	2.0 a
Goat weed + Poultry manure	13.8 b	1.9 a
Elephant grass + Cow dung	13.3 b	1.6 a
Elephant grass + Poultry manure	4.1 b	1.5 a
Sunflower + Poultry manure	7.5 b	1.3 b

\*Means within a column followed by the same letter are not significantly different according to SNK test at 5%.

### Regression of physicochemical properties on disease suppression

Regression showed that, physicochemical properties significantly influenced bacterial wilt severity (Table 4). The regression was not significant for Na and Mg. A significant regression was observed on N, pH, Ca, K, OM and C/N. High disease severity was observed on plants grown on substrate with low physicochemical properties; for example: plants grown on pure soil had the highest disease severity.

**Table 4:** Regression of physicochemical properties of compost on disease severity

Property	Slope	Intercept	R <sup>2</sup>	Probability
N	-1.67±0.054	3.658±0.562	0.659	0.027*
pH	-2.129±0.764	17.656±5.621	0.608	0.039*
Ca	-0.48±0.013	3.430±0.412	0.739	0.013*
K	-6.49±0.434	3.731±0.406	0.804	0.006**
Mg	-0.0240±0.10	2.774±0.308	0.521	0.067ns
OM	-2.2390±0.060	5.246±0.823	0.762	0.010**
Na	-2.6093±0.926	2.361±0.610	0.081	0.536ns
C/N	-0.167±1.2	3.658±1.120	0.408	0.122*

\*\* : significant ( $p \leq 0.01$ ), \* : significant ( $p = 0.05$ ), Ns: not significant ( $p > 0.05$ ), R<sup>2</sup> = coefficient of determination.

### Relation between microbial population and disease suppression

A high disease severity was observed on soil and the least on compost made with sunflower and poultry manure. Soil also recorded the least number of microbial colonies, while sunflower + poultry manure recorded the highest value. The regression was not significant. The slope was negative ( $-0.008 \pm 0.004$ ) showing that an increase in the population of fluorescent bacteria resulted in a decrease in disease severity.

### Discussion

Soil texture, temperature, organic matter content, dissolved organic carbon content, pH and microbial communities are among the factors affecting the survival of *R. solanacearum*

[9]. The pH in sterilized soils was slightly neutral, compared to the pH of water used for multiplication of the bacteria. The organic matter content provided a favorable medium for the proliferation of microorganisms, as well as providing nutrients. Certain bacteria like *Pseudomonas fluorescens* and actinomycetes have been found to delay the development of bacterial wilt, and reduce its incidence [17]. The variation in response to inoculation can therefore be attributed to differences in the microbial communities in the various substrates, as indicated by the microbial count. Organic matter has been used to improve soil structure, fertility and yields in many crop systems [18, 19, 20], and to decrease the incidence of diseases caused by soil pathogens [20]. It was shown that amendments of cow dung and poultry manure reduce the incidence of both bacterial wilt caused by *R. solanacearum* and *Fusarium oxysporium* f.sp. *lycopescici* [21]. Variation in incidence and severity on plants grown on the different compost types in our trial can be attributed to differences in the physicochemical and biological properties. In fact efficient soil management generally improves the composition and activity of soil microbiota thereby enhancing the biological control capacity of the soil [9]. Soils with low organic matter content and microbial activity tend to be conducive to root diseases of plants. This was the case of compost type made with sunflower + poultry manure with the highest population of micro organisms and organic matter content, compared with sterilized soil. Hence the high microbial population correlates the low bacterial wilt incidence and severity on the sunflower + poultry manure compost, compared with the sterilized soil. Pathogens thrive in such mineralized soils in the presence of the host plant; hence plants grown on soils recorded the highest disease severity and incidence [22]. In addition, there are many variables that affect the degree and duration of biological disease suppression taking place when compost is used. Variables include the type of compost feedstock, types of microbes that colonize the compost, soil/media characteristics, and compost characteristics (level of stability, salinity, nutrients) [23]. The nutrition of a plant determines in large measure its resistance or susceptibility to disease, its virulence or ability of pathogens to survive. Mineral nutrients, in many situations, are the first line of defense against plant diseases [24]. One of the important elements needed in potato production is Potassium. It has a role to play in resistance to diseases and improved storability, among other functions [25]. Soil analysis has lowest value of components compared to composts. This can also be one of the reasons behind the high severity and susceptibility observed on plants grown on these soils. Potassium deficiencies impair the crop's resistance to diseases and its ability to tolerate stresses such as drought [26]. Chemical analysis of the substrates revealed that compost made with sunflower + poultry manure had the highest amount of Ca, followed by compost made with elephant grass + poultry manure. These two types of composts gave the best result on disease suppression. In fact, it has been proved that calcium nutrition reduces the incidence of wilt in the soil [21]. The variation in the C/N observed also contributed to differences in the available nitrogen content thereby inducing varying microbial activities. This also explains the variation in the microbial population densities in the different composts as well as variation in disease expression. This correlates with the high C/N ratio in compost made with sunflower. The suppressive effect of organic matter on the pathogen is attributed to a shift in microbial population activities in the

soil [24]. The pH (H<sub>2</sub>O) was lower in soil samples. The bacterium can survive for days to years in water, wet soils or deep soil layers [27]. This pH was close to the pH of water, which contributed to the survival of the bacteria in sterilized soils. The different compost types therefore had better conditions for microbial activities than pure soils. Manure does not cause a breakdown in the pathogenicity of the pathogen. Rather, it induces tolerance of the stem vessels of the host plant [21].

### Conclusion

The objective of this study was to increase potato production through an integrated disease management program by using compost. The most suppressive compost was that made with *T. diversifolia* + poultry manure where Fluorescent bacteria were significantly concentrated (20x 10<sup>11</sup>cfu/ml). Incorporation of compost into the soil significantly contributed to suppress bacterial wilt incidence and severity. Physicochemical elements such as N, Ca, K, Mg, as well as OM and OC content were negatively correlated with disease severity. The microbial population shows a positive correlation with wilt severity. Compost made of Sunflower + poultry manure has the highest microbial population density. Bacterial wilt severity was lower in all the compost types compared to the control. These results suggest that compost mixture with poultry manure and cow dung could be incorporated to an integrated management of bacterial wilt of potato.

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