Impact of plant extracts and organic amendments on the growth of *Ralstonia solanacearum* and severity of potato bacterial wilt

Fontem D.A. and N'tchorere B.M.J.

Faculty of Agriculture, University of Dschang, Box 208, Dschang, Cameroon. Email: dfontem@yahoo.com

Abstract

Continuous potato (*Solanum tuberosum*) production in the tropics and subtropics is usually handicapped by bacterial wilt incited by *Ralstonia solanacearum.* Laboratory and screen house experiments were conducted to assess antibactericidal activities of plant extracts and organic amendments respectively on pathogen growth and bacterial wilt severity. Antibacterial activity of the extracts was determined by colonial growth on potato sucrose agar medium amended with various concentrations of the extracts. Potato (cv Cipira) tubers were planted Dschang and Foumbot in 10% organic amended soils and inoculated 30 days later with 10 ml of 10^7 cfu/ml of the bacterial suspension. Data on wilt severity and tuber production were recorded. Methanol leaf extracts of *Crotalaria falcata* and *Tephrosia vogelii* were more active (ED_{90} = 1.40–5.94 mg/ml) than the corresponding acetone extracts (ED_{90} = 47.60–77.14 mg/ml), while the reverse was observed for acetone leaf extracts of *Brassica integrifolia* and *Cissus aralioides*. All organic amendments significantly reduced bacterial wilt severity in both sites. Significant (P = 0.001) negative correlations were observed between bacterial wilt severity and tuber yields. Crotalaria and Tephrosia amendments were more effective in the reducing wilt severity and increasing tuber yield than Brassica or Cissus amendments. Results indicate a potential of plant extracts in bacterial wilt management and a necessity for an adoption of integrated *R solanacearum* management strategies through a judicious use of organic amendments in potato production.

Keywords: Potato, bacterial wilt, organic amendments, plant extract, *Ralstonia solanacearum.*

Introduction

Bacterial wilt caused by the soil borne vascular pathogen, *Ralstonia solanacearum* (Smith) Yabuuchi *et al.*), is a devastating disease in both tropical and sub tropical regions, infecting over 200 plant families. Important host crops include the *Solanaceae* (e.g. potato, tomato, eggplant, garden huckleberry, tobacco), *Musaceae* (e.g. bananas), *Papilionaceae* (e.g. groundnuts) and *Zingiberaceae* (e.g. ginger) (Hayward, 1991). Thus, it is a major limitation to a sustainable production of a wide range of crops in Sub-Sahara Africa (Adipala *et al.,* 2001). Losses of up to 100% were reported for potato and tomato in the early 1970s in Uganda (Simbwa-Bunya, 1971).

In potato, bacterial wilt severity is often exacerbated by low fertility caused by intensive or continuous cultivation and use of infected seed tubers. Control has been difficult due to the high variability of the pathogen, its extremely wide host range, lack of possibility for chemical control and high ability of the pathogen to survive in diverse environments. The use of resistant varieties is the simplest and most effective method to control any disease. However, bacterial wilt resistance is overcome by the genetic diversity of the pathogen as well as genotype x environment interactions.

Incorporation of composts into soils is a fundamental cultural practice in crop production. Organic matter is usually incorporated into the soil for sustainable crop production, because it enhances soil fertility through modification of soil physical, chemical and biological properties (Islam and Toyota, 2004). Moreover, the use of organic amendments is a widespread means to control diseases caused by soil-borne plant pathogens (Huang and Huang, 1993). The effects of organic amendments on the severity of bacterial wilt have been reported in several countries, including Kenya (Muriithi and Irungu, 2004), Nigeria (Adebayo & Ekpo, 2001), Uganda (Lemaga *et al.*, 2001) and Taiwan (Akew *et al.*, 1996). However, few studies address the *in vitro* effects of plant extracts on bacterial growth and the impact of their amendments on bacterial wilt severity. This study was designed to investigate the effects of plant extracts and organic amendments respectively on the *in vivo* growth of *R. solanacearum* and the severity of bacterial wilt.

Materials and methods

Plant and bacterial materials. Fresh leaves of wild cabbage *(Brassica integrifolia* West) Rupr.), cissus (*Cissus aralioides* (Baker) Planch.)*,* crotalaria (*Crotalaria falcata* Vahl ex DC) and tephrosia (*Tephrosia vogelii* Hook. f.) were collected in Dschang, western highlands of Cameroon during September to November 2004. These plants are annual weeds that invade cultivated fields and also grow wild on fallow lands. Laboratory and greenhouse experiments were carried out to assess their antibacterial activities on potato bacterial wilt pathogen, *R. solanacearum*.

The bacterium, *R. solanacearum*, was isolated from infected potatoes in Dschang and cultured on potato sucrose agar (PSA). The strain belonged to race 3. The bacterial suspension was prepared in sterile distilled water after 48 h and adjusted to 10^7 colony forming units (cfu)/ml (OD=0.3 at 600 nm) using a Pye Unicam SP6-450 UV/VIS spectrophotometer.

Extraction and bioassay of the extracts. The collected plant leaves were dried at 30 °C for 7 days. The dried leaves were ground to powder and macerated at room temperature with methanol or acetone for 5 days. The combined methanol or acetone extracts were concentrated to dryness using a rotary vacuum evaporator at 65 °C for methanol and 56 °C for acetone. The yields of the extracts were expressed in g/100 g dry weight.

The antibacterial activity of the extracts was determined by colonial growth on potato sucrose agar (PSA) medium amended with various concentrations of the extracts. The required amount of extract was dissolved in 5% DMSO and mixed with the agar medium to produce concentrations of 1, 10, 100, 1000 mg/ml. Control plates contained the same treatment without any extract. Petri plates containing various concentrations of the extracts or control were streak inoculated with 0.1 ml of 10^3 cfu/ml of the bacterial suspension. There were five plates per treatment and the experiment was repeated twice. Inoculated plates were incubated at 30 °C in dark for 24 h. The number of colonies were counted for each plate and percent growth inhibition was determined using the formula: $(N_u - N_a)100/ N_u$, where N_u and N_a are the number of cfu on the unamended and amended PSA medium, respectively.

Effect of organic amendments on bacterial wilt severity. This trial was carried out at the screen house in Dschang (1400 m) and Foumbot (1000 m) during April to August 2005. The soil types of both sites were nitrosol (pH-H_2O = 6.8, 6.5% OM, 13-14% C/N, 23 % clay) for Dschang and andosol (pH-H_2O = 6.4, 12.9% OM, 12.1% C/N, 15 % clay) for Foumbot. Dschang and Foumbot are respectively major potato and tomato production regions of Cameroon. Experiments were designed in a randomized complete blocks with four replicates. A total of 100 pots each filled with 450g of soil were used, five pots for each treatment. Pots were amended by adding 50 g of fresh shoots of *B. integrifolia*, *C. aralioides, C. falcata* or *T. vogelii.* The amended combination was allowed to decompose for four weeks. Unamended pots served as controls and the trial was repeated once.

After amendment, potato (cv. Cipira) tubers, obtained from a certified commercial supplier, were planted one tuber per pot into each pot. Plants were inoculated 30 days after planting by pouring 30 ml of 10^7 cfu/ml round the base of each seedling. Each plant was watered daily with 30 ml of sterile distilled water.

Data on wilt severity and tuber production were collected. Wilt severity was assessed weekly using a 1-5 scale (Adebayo & Ekpo, 2001) in which 1 = no symptom, 2 = one leaf at least partially wilted, 3 = two or three leaves wilted, 4 = four or more leaves wilted and 5 = plant dead. Healthy tubers were collected and weighed 94 days after planting in both locations and yields were expressed in g/plant.

Data analysis. For *in vitro* tests, percent bacterial growth inhibition was transformed into probits and the values obtained were regressed on the logarithm of the concentration of the extracts. The equivalent concentrations for 90 % inhibition (EC_{90}) of bacterial growth were calculated for each extract as suggested by Finney (1971). All data were subjected to analyses of variance and Duncan's multiple range test (P = 0.05) was used to compare treatment means.

Results

In vitro experiments. The extraction of the various plant materials yielded 10.51 – 17.35 g/100g for methanol extracts and 9.13 – 14.93 g/100 g for acetone extracts. Consequently, for all plant materials used, methanol yielded relative higher extract than acetone. The colour of the extracts varied with the solvent used (Table 1).

Table 1. Yield and colour characteristics of plant extracts used for *in vitro* tests

Extract	Yield (g/100 g dry wt)		Colour	
	Methanol	Acetone	Methanol	Acetone
Crotalaria falcata	11.16	9.87	Green	Deep brown
Tephrosia vogelii	15.78	13.57	Green	Green
Brassica integrifolia	17.35	14.93	Brown	Deep brown
Cissus aralioides	10.51	9.13	Brown	Green

The extracts tested had adverse effects on the growth of the bacterium at different concentrations. Bacterial growth reduced with increase in concentration of each extract. Bactericidal activities of the extracts were rated in terms of EC_{90} values. The antibacterial activity of the extracts depended on the solvent used. Methanol extracts of Crotalaria and Tephrosia were more active (EC_{90} = 1.40 – 5.94) than the corresponding acetone extracts (EC_{90} = 47.60 – 77.14) while the reverse was observed for Brassica and Cissus extracts. The EC_{90} values for Crotalaria and Tephrosia methanol extracts were significantly (P = 0.01) lower than those of the Brassica and Cissus extracts tested, while the reverse trend was observed for acetone extracts. Based on EC_{90} values, Crotalaria and Tephrosia methanol extracts and Brassica acetone extracts were highly active on the bacterium (Table 2).

Table 2. Bactericidal activity (ED_{90}) of methanol and acetone plant extracts on *in vitro* growth of *R. solanacearum*

Extract	Methanol (mg/ml)	Acetone (mg/ml)
Crotalaria falcata	5.94 c	47.60 b
Tephrosia vogelii	1.40 c	77.14 a
Brassica integrifolia	62.87 b	6.12 d
Cissus aralioides	354.15 a	25.58 c

[z]Means in a column followed by the same letter are not significantly different according to Duncan's multiple range test (P = 0.05).

Effect of organic amendments on bacterial wilt severity. Bacterial wilt severity differed with location and soil treatments. All plant amendments significantly reduced wilt severity in both locations. Crotalaria and Tephrosia amendments were more effective in reducing disease severity than Brassica or Cissus amendments (Fig 1). The least wilt severities in both locations were obtained in pots amended with Crotalaria and Tephrosia leaves (Table 3).

Potato tuber yields were significantly higher in amended soils compared to the control. The yields were increased by 48-207% in Dschang and 206-745% in Foumbot. The increases were consistently higher in Foumbot than in Dschang. In both locations applications of Crotalaria or Tephrosia amendments consistently produced higher tuber yields compared to Brassica or Cissus amendments (Table 4). Tuber yields decreased with increase in wilt severity in both locations. Regression analyses showed significant (P = 0.001) negative correlations between bacterial wilt severity (x) and tuber yields (y). The regression equations were y = 383.11-62.20x (R^2 = 0.98) for Dschang and y = 344.55-60.17x (R^2 = 0.98) for Foumbot (Fig. 2).

Table 3. Severity of potato bacterial wilt as affected by organic amendments in two locations recorded 57 days after inoculation

Amendment	Dschang	Foumbot
Control	5.0 a[z]	5.0 a
Crotalaria falcata	2.4 cd	1.9 c
Tephrosia vogelii	2.2 d	1.2 c
Brassica integrifolia	4.0 b	4.4 a
Cissus aralioides	3.0 c	3.4 b

[z]Means in a column followed by the same letter are not significantly different according to Duncan's multiple range test (P = 0.05).

Table 4. Tuber yields (g/plant) of potato as affected by organic amendments in two locations recorded 64 days after inoculation

Amendment	Dschang	Foumbot
Control	82 c[z]	31 c
Crotalaria falcata	237 (189) a	242 (681) a
Tephrosia vogelii	252 (207) a	262 (745) a
Brassica integrifolia	121 (48) bc	95 (206) bc
Cissus aralioides	191 (133) ab	136 (338) b

[z]Means in a column followed by the same letter are not significantly different according to Duncan's multiple range test (P = 0.05). Values in parentheses are percent increases over the unamended control

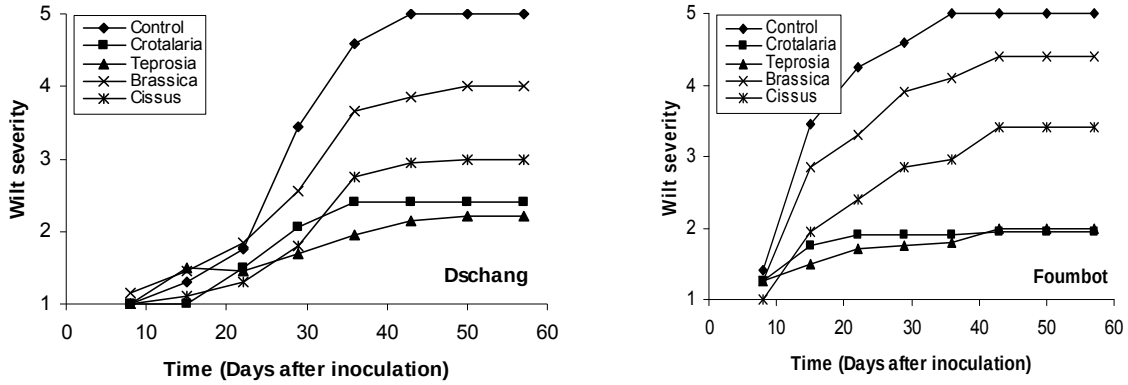

Figure 1. Effect of plant amendments on the progress of potato bacterial wilt severity in two location of Cameroon.

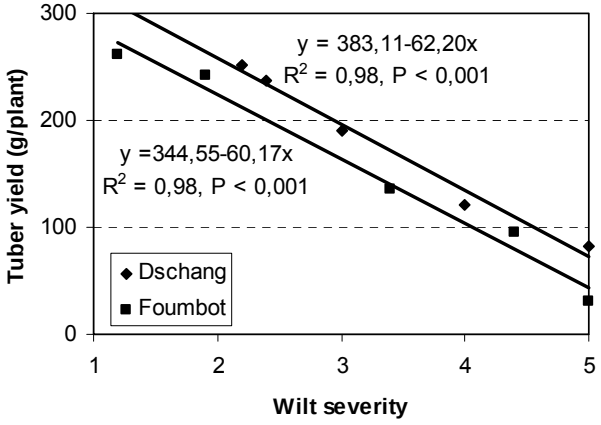

Figure 2. Relationship between bacterial wilt severity and potato tuber yield in two locations

Discussion

This study demonstrates the *in vitro* antibacterial effects of plant extracts and the *in vivo* effects of their amendments. Several studies have reported the *in vitro* antibacterial effects of some herbs (Satish *et al.,* 1999; Vudhivanich, 2003). As the bacterium is known to be transmitted through seeds, one important application of plant extracts is as a seed protectant.

Organic amendments significantly improved tuber yields in both sites, suggesting their importance in bacterial wilt management and plant production. In East Africa, some researchers (Lemaga *et al*., 2001; Muriithi and Irungu, 2004) also reported significant reductions of bacterial wilt incidence with improvements in potato yields following a combined application of organic and inorganic soil amendments. The effects of Crotalaria green manure on bacterial wilt control has also been reported (Akew *et al*., 1996; Adebayo and Ekpo, 2001).

The difference in wilt severity between both sites could be attributed to soil organic matter content as the organic matter content of the soil used in Foumbot was twice as high of that used in Dschang. Moffet *et al*. (1983) reported that bacterial wilt incidence reduces in soils with high organic matter content. There appears to be an indirect effect of organic amendment on pathogen population in the soil. Some researchers (Michel and Mew, 1998; Islam and Toyota, 2004) reported that organic amendments enhance antagonist activity in the soil while suppressing soil borne pathogens. Moreover, applications of these amendments increase soil pH and nitrate accumulation and reduce the C/N ratio that is necessary for microbial antagonists. Islam and Toyota (2004) did not observe any disease control in autoclaved amended soils and concluded that indigenous microorganisms in condusive soils rather than those in the compost play an important role in the suppressive effect.

In vitro results with methanol extracts were similar to screen house tests. Crotalaria and Tephrosia leaf extracts were more active *in vitro* than those of Brassica and Cissus and results of organic amendments followed a similarly trend. However, further studies are needed to confirm the use of *in vitro* tests in the screening of possible antibacterial organic amendments. This study shows that the four plant extracts used may be developed as effective antibacterial compounds. Moreover, Crotalaria or Tephrosia organic amendment induces a higher antibacterial activity on *R. solanacearum* than Brassica or Cissus amendment. Consequently, the former organic amendments could be envisaged as possible components of IPM tools for bacterial wilt.

References

Adebayo O.S.I.; Ekpo, E.J.A. 2001. Effects of organic amendments on tomato diseases caused by *Ralstonia solanacearum* and *Fusarium oxysporum f.sp. lycopercici*. African Crop Sci. Conf. Proc.5: 305-307.

Adipala, E.; Tusiime, G. ; Patel, B.K. ; Lemaga, B.; Olanya, M. 2001. Bacterial wilt management: a dilemma for Sub-Sahara Africa. African Crop Sci. Conf. Proc. 5: 433-437.

Akew, S.; Trevorrow, P.R.; Kirkegard, J. 1996. Mustard green manure reduces bacterial wilt. Bacterial Wilt Newsletter 13: 5-6.

Finney, D. F. 1971. Probit Analysis. 3rd Ed. University Press, Cambridge. 333 p.

Hayward, A.C. 1991.Biology and epidemiology of bacterial wilt caused by *Pseudomonas solanacearum*. An. Rev. Phytopath. 29:65-87.

Huang, H. C.; Huang, J. W. 1993. Prospects for control of soilborne plant pathogens by soil amendment. Curr. Topics Bot. Res. 1:223-235.

Islam, T.M.D.; Toyota, K. 2004. Suppression of bacterial wilt of tomato by *Ralstonia solanacearum* by incorporation of composts in soil and possible mechanisms. Microbes Environ. 19:53-60.

Lemaga, B.; Siriri, D.; Ebanyat, P. 2001. Effect of soil amendments on bacterial wilt incidence and yield of potatoes in Southwestern Uganda. African Crop Sci. J. 9: 267-278.

Michel, V.V.; Mew, T.W. 1998. Effect of a soil amendment on the survival of *Ralstonia solanacearum* in different soils. Phytopathology 88: 300-305.

Moffet, M.L.; Giles, J.E.; Wood, B.A. 1983. Survival of *Pseudomonas solanacearum* biovars 2 and 3 in soil: effect of moisture and soil type. Soil Biol. and Biochem.15: 587-591.

Muriithi, L.M.M.; Irungu, J.W. 2004. Effect of integrated use of inorganic fertilizer and organic manures on bacterial wilt incidence (BWI) and tuber yield in potato production systems on hill slopes of Central Kenya. J. of Mountain Science 1*:* 81-88.

Satish, S.; Raveesha, K.A.; Janardhana, G.R. 1999. Antibacterial activity of plant extracts on phytopathogenic *Xanthosoma campestris* pathovars. Letters in Appl. Microbiol. 28:145-147.

Simbwa-Bunya, M. 1971. Resistance of groundnut varieties to bacterial wilt (*Pseudomonas solanacearum*) in Uganda. East African Agriculture and Forestry Journal 37: 341-343.

Vudhivanich, S. 2003. Potential of some Thai herbal extracts for inhibiting growth of *Ralstonia solanacearum*, the causal agent of bacterial wilt of tomato. Kamphaengsaen Acad. J. 1(2): 70 -76.