EFFECTS OF DIFFERENT SOIL AMENDMENTS ON BACTERIAL WILT CAUSED BY *RALSTONIA SOLANACEARUM* AND ON THE YIELD OF TOMATO

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SUMMARY

Ralstonia solanacearum race 3 biovar 2 (phylotype II) is the causal agent of bacterial wilt of tomato, the most destructive bacterial disease of this crop in Ethiopia for which no effective control measures are available. In this study, the effects of amending topsoil with three different levels (1, 5 and 10%) of cocopeat, farmyard manure (FYM) compost and green compost, and two levels of bacterial inoculations were tested on infection of tomato by *R. solanacearum* compared to non-inoculated treatments. Non-amended topsoil with and without *R. solanacearum* were included as control treatments. Survival of and infection by *R. solanacearum* and yield-associated agronomic responses were used for evaluation along with physico-chemical and biological characteristics of amended soils. Amendments resulted in changes in physico-chemical properties (such as electrical conductivity, organic matter content, total carbon, dissolved organic carbon, NH_4^+, NO_3^-) and microbial activity of the amended soil and the effects were found to be higher at the higher rate of application. Effects on disease suppression and survival of the pathogen in the soil differed depending on amendment type and application rate. Higher disease severity was recorded in soil amended with 10% green compost compared to the control treatment. Complete suppression of *R. solanacearum* was observed in pots amended with 5 and 10% farm yard manure (FYM), 1% green compost and 10% cocopeat. Absence of disease at the highest rate of FYM was supported by a lower number of culturable *R. solanacearum* bacteria recovered from rhizosphere soil two months post-inoculation in soil amended with 10% FYM. Soil amended with 10% FYM gave higher root and above-ground dry weight. Moreover, FYM added to topsoil at 5 and 10% gave significantly higher above-ground fresh weight. This study indicated that amending topsoil with different types and rates of amendment can suppress bacterial wilt severity and pathogen survival in the soil. Amendments also enhanced tomato yield, the higher rates of amendments being the most effective except green compost at 10% which gave a 27% lower yield compared with the higher rate of FYM. Among the amendments tested, FYM at 5 or 10% would be an interesting option to manage *R. solanacearum* in the major tomato-growing regions of Ethiopia. However, the mechanisms of disease suppression at higher rates of FYM need to be investigated.

Key words: soil suppressiveness, compost, bacterial wilt of tomato, soil-borne disease, control.

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INTRODUCTION

Ralstonia solanacearum [(Smith) Yabuuchi *et al.* (1995)], is one of the most important and widely distributed plant pathogenic bacteria in the tropical, sub-tropical and warm temperate climates of the world (Hayward, 1991). It causes bacterial wilt disease on over 200 plant species in 50 families (Hayward, 2000) and remains the major biotic factor limiting growth and development of several important crops of family Solanaceae, including potato, tomato, eggplant, pepper and tobacco (French and Sequeria, 1970; Anith *et al.*, 2004) and Leguminosae (e.g. ground nut and French bean), and several tree and shrub hosts (Genin and Boucher, 2002). The strains of *R. solancearum* have been subdivided into five races on the basis of host range (He *et al.*, 1983; Hayward, 1991) and into six biovars based on utilization of three disaccharides and three hexose alcohols (He *et al.*, 1983). Unlike races 1, 2, 4 and 5 (EPPO, 2004) with optimum temperatures of around 35°C, race 3 biovar 2 (phylotype II) has a temperature optimum of 27°C. Race 2 occurs mainly in tropical areas of South America and is pathogenic to triploid banana, whereas race 3 occurs at higher elevations of sub-tropical and warm temperate regions and attacks potato, tomato and pepper among the other hosts (EPPO, 2004). Race 4 is pathogenic to ginger, while race 5 biovar 5 (EPPO, 2004) is specialized on mulberry.

Tomato (*Lycopersicon esculentum* Mill), is one of the most important and widely grown crops in Ethiopia. Altitudes between 700 and 2,000 metres characterized by

warm, dry days and cooler nights favour optimum growth and development of tomatoes (Lemma, 2002). However, productivity is very low with average national yields of about 4.45 ton ha^{-1} (MoARD, 2005). Bacterial wilt of tomato (BWT) is the most important soil-borne disease of this and other solanaceous crops in Ethiopia (Yaynu, 1989; Lemessa and Zeller, 2007a) and its effect is more pronounced during the dry season (Lemessa and Zeller, 2007a). Incidences of BWT as high as 55% were reported in the major tomato-producing regions of Ethiopia (EARO, 2002), where cost-effective control is lacking.

Various control strategies have been suggested such as crop rotation, bio-fumigants, use of resistant cultivars, grafting on resistant rootstocks, disinfection of plant materials (Hsu, 1991), microbial antagonists (Lemessa and Zeller, 2007b; Messiha *et al.*, 2007a) and biological soil disinfestation (Messiha *et al.*, 2007a). This last is the only effective method available to date. However, it is not a good option for resource-poor farmers owing to its high cost. As a result, the disease is still a major problem causing losses to tomato production due to its wide host range and excellent survival in soil and water (Hsu, 1991). Alternative management options are therefore strongly needed.

One of these options is the amendment of soils with different organic materials. Amending agricultural soils and soilless growing media with organic matter supplies plant nutrients, increases natural suppressiveness of the soil against soil-borne pathogens and improves physico-chemical and biological characteristics (Veeken *et al.*, 2005; Janvier *et al.*, 2007). On the other hand, organic matter inputs (Yamulki, 2006) can lead to negative effects such as temporary oxygen depletion and denitrification, nitrogen immobilization and plant pathogen stimulation. Thus, knowing the type and optimum rate of organic amendment that results in positive effects on disease suppression and plant growth is of utmost importance to the farmers.

The suppressive effects of organic amendments on the survival of *Ralstonia* vary with soil types (Michel and Mew, 1998; van Elsas *et al.*, 2000). Soil texture, temperature, organic matter content, pH, microbial communities, moisture content (van Elsas *et al.*, 2005) and dissolved organic carbon (DOC) content (Messiha *et al.*, 2007b) are among those factors affecting the survival of *R. solanacearum* race 3 biovar 2. Efficient soil management generally improves the composition and activity of soil microbiota thereby enhancing the biological control capacity of the soil (Chellemi *et al.*, 1992; van Elsas *et al.*, 2005). Therefore, soil amendments with organic materials could be an indirect way of stimulating biocontrol in the soil. Frequent application of organic materials such as manure or compost (Hoitink and Boehm, 1999) eventually results in higher substrate availability for competitors, reducing the growth of pathogens in the rhizoshere and reducing their infection rate.

Incorporation of household compost (Schönfeld *et al.*, 2003), cow dung manure (Nishyama, *et al.*, 1999) and pig slurry (Gorissen *et al.*, 2004) have been found to reduce bacterial wilt incidence and severity. For instance, soil amended with organic materials, inorganic materials (NPK fertilizers) or different combinations of these amendments considerably affected bacterial wilt incidence on potato and increased yield (Lemaga *et al.*, 2005). Islam and Toyota (2004) reported suppression of BWT in soils amended with poultry manure and farmyard manure. Suppression of bacterial wilt by pig slurry (Gorissen *et al.*, 2004) was associated with a microbial community shift.

Ammonia toxicity could also be one mechanism of suppression (Michel and Mew, 1998). Cocopeat, a by-product of the coconut industry, is a perfect hydroponic medium for several vegetables and ornamental cut flowers. The suppressive ability of cocopeat on certain soil-borne diseases has been reported (Candole and Evans, 2004). According to these authors, *Phytophthora capsici*, *P. nicotianae*, *Pythium aphanidermatatum* and *P. ultimum* severity was reduced by 76, 80, 32 and 11% respectively in cocopeat media compared to peat moss. *In vitro* suppression of cocopeat to certain soil-borne pathogens is mainly due to microorganisms associated with the substrate (Hyder *et al.*, 2009). On the contrary, the inability of cocopeat to suppress *Fusarium* wilt of carnation was reported by Borrero *et al.* (2009). Research examining the suppressive effect of cocopeat on *R. solanacearum* is rather limited.

There is little information regarding the comparative effect of different organic amendments in suppressing BWT and recommendations are lacking as to the type and rate of organic amendments to be used for management. Therefore, the objectives of this study were to: (i) explore the suppressive ability of farmyard manure compost, green compost and cocopeat amendments at different rates on bacterial wilt and yield of tomato; (ii) evaluate the effects of farmyard manure compost, green compost and cocopeat amendments at different rates on physico-chemical and microbial characteristics of the amended topsoil.

MATERIALS AND METHODS

Site description and experimental design. Two tomato bioassays were conducted between September 2007 and January 2008 in a lath house at Jimma University College of Agriculture and Veterinary Medicine (JUCAVM), Jimma, Ethiopia, located at 7^0, 33'N and 36^0, 57'E at an altitude of 1710 m. Physico-chemical characteristics and microbial activity of the amendments and the amended soils were analyzed at the Laboratory of

Biological Farming Systems Groups, Wageningen University, The Netherlands.

Two experiments were conducted simultaneously. Experiment 1 was designed to determine the effects of amendments on bacterial wilt incidence and severity, while experiment 2 was designed to assess the effects of amendments on survival of *R. solanacearum* populations in rhizosphere soil. Three-factor factorial design in 7 and 3 blocks was used in experiments 1 and 2, respectively. Three organic amendments, namely cocopeat, farmyard manure (FYM) compost and green compost, were used to amend topsoil. Each amendment was added to infested and non-infested topsoil at three rates (1, 5, and 10% v/v). Non-amended soil was used as control. Cocopeat was obtained from AXRUM Company in Addis Ababa, Ethiopia. The other two organic amendments (FYM and green compost) were prepared at the composting site of JUCAVM. Cow dung manure and Tef straw were the compost ingredients for FYM while the compost ingredients of green compost were coffee husks and grass. In both cases, the compost ingredients were mixed in equal volumes and composted to full maturity for 3 months following the common method practiced by farmers. Compost piles were turned twice at four-week intervals. Topsoil was dug from 0-20 cm depth from a location known to be free from *R. solanacearum* and transported to JUCAVM. The soil was sieved using a 2-mm screen to remove soil clumps and roots before use. Three-liter pots with drainage holes at the bottom were used as experimental units.

Inoculum preparation and inoculation. *R. solanacearum* isolate PPRC-T60 Rs used to infest the media was obtained from the National Plant Protection Research Center (PPRC), Ambo, Ethiopia. The strain was isolated from tobacco and known to be race 3 biovar 2 on the basis of host range and biochemical utilization (Nasir Aliye, personal communication). For inoculation, the bacterium was grown on triphenyl tetrazolium chloride (TZC) agar medium (Kelman, 1954) at 28°C for 48 h and wild-type bacterial colonies (based on colony morphology) were harvested and suspended in casamino acids, peptone and glucose (CPG) liquid culture and grown for three days at room temperature. Cultures were centrifuged at 10,000 rpm for 10 min at 10°C. Bacterial pellets were suspended in distilled water and adjusted to 10^9 CFU ml^{-1}. Then, about 2/3 of the potting soil mixes were taken out from the pot and thoroughly mixed with 75 ml of inoculum to have a final bacterial density of 10^8 CFU ml^{-1} (*ca.* $5x10^6$ CFU g^{-1} dry soil). The control treatments were inoculated with an equal volume (75 ml) of distilled water to maintain the moisture content of the media as uniform as possible.

Seedlings of tomato cv. Heinz 1370 obtained from Melkassa Agricultural Research Center (Melkassa, Ethiopia) were grown following standard procedure in a field nursery. Two-week old seedlings were transplanted into each pot within 1 h after bacterial inoculation. Seven pots per treatment with two plants each were used for the disease suppression experiment, while three pots with two plants per pot were set aside for the *R. solanacearum* survival experiment. Watering was done using a hose with fine nozzles and pots were watered independently. A distance of 15 cm between pots was maintained to avoid cross-contamination during watering.

Populations of *R. solanacearum*. The population density of *R. solanacearum* in rhizosphere soil was assessed one week and two months post-planting. Bacterial population density was determined from stem pieces obtained from the base of symptomless plants two months post-transplanting. To determine the population, one sample (100 g) per pot and two samples per treatment were collected. The culturable population of the pathogen was determined by the dilution plate method. Starting with 10 g sub-sample, serial dilutions of each sample were made in distilled water, and two suitable dilutions per sample were selected for plating. Duplicate (pseudo-replication) 50 µl aliquots of the two suitable dilutions were plated on modified semi-selective media South Africa (SMSA) (Anonymous, 1998) and incubated at 28°C. The antibiotics used in SMSA media included 1% polymyxin B sulphate, 1% bacitracin, 0.1% penicillin, 1% chloramphenicol and 1% cycloheximide. The bacterial colonies were counted after 6 days incubation and the number of CFUs per gram of wet soil was computed. Measurements for efficacy of the media at time zero was not taken.

Chemical, physical and biological analysis of topsoil and organic amendments. Plant available N-NO_3 and N-NH_4 were quantified according to Houba *et al.* (2000). Samples were extracted in 0.01 M $CaCl_2$ and analyzed using a segmented-flow system (Auto-analyzer II, Technicon, UK). For determination of available P content, soil samples were extracted in 0.01 M $CaCl_2$ and analyzed spectrophotometrically (Novozamsky *et al*., 1984) using a segmented–flow system (Skalar Analytical BV, The Netherlands). Total N and organic C contents were determined using the Dumas Method with a CHN1110 Element Analyzer (CE Instruments, Italy). Available K was determined after sample extraction with 0.01 M $CaCl_2$ and vaporization, and was analyzed by flame emission spectrophotometer at a wavelength of 766.5 nm (Houba *et al.*, 1989b). The pH was measured after 0.01 M $CaCl_2$ extraction using a pH/mV meter with a combined electrode (Houba and Novozamsky, 1998). Dissolved organic carbon (DOC) and total soluble nitrogen (Nts) were determined using a TOC-VCPH/TOC-VCPN total organic carbon analyzer (Shimadzu, Japan). Dissolved organic nitrogen (DON) was re-calculated from total dissolved nitrogen, NO_3-N and NH_4-N as: DON = Nts- (NO_3-N +

NH_4-N). Organic matter was determined by loss-on-ignition, i.e. by dry combustion of the organic material in a furnace at 500-550°C so that the weight loss indicated the content of organic matter in the sample (Houba *et al.*, 1997).

Electrical conductivity (EC) was determined according to ISO 7888-1985 (water quality-determination of electrical conductivity) and ISO 3696-1987 (water for analytical laboratory use - specification and test methods).The C/N ratio was calculated from the total carbon and nitrogen contents.

Oxygen uptake rate (OUR) of each sample was measured using the OxiTop system (WTW, Germany) described in Grigatti *et al.* (2007). This system determines pressure change that coincides with microbial use of oxygen in closed 2 litres glass bottles with CO_2 trap (soda lime) in the head space. About 10 g sample was put in each bottle, followed by the addition of 180 ml of demineralized water, 10 ml of pH 7 phosphate buffer (43.08 g l^{-1} KH_2PO_4, 88.86 g $l^{-1}Na_2HPO_4$ x $2H_2O$, 1 l H_2O), 10 ml of macronutrients (4.31 g l^{-1} NH_4Cl, 5.39 g $l^{-1}CaCl_2$ x $2H_2O$, 4.31 g $l^{-1}MgSO_4$ x $7H_2O$, 54 mg l^{-1} $FeCl_3$ x $6H_2O$, 1 l H_2O), and 0.2 ml of micronutrients [2 g l^{-1} $FeCl_3$ x $4H_2O$, 2 g $l^{-1}CoCl_2$ x $6H_2O$, 0.5 g l^{-1} $MnCl_2$ x $4H_2O$, 30 mg $l^{-1}CuCl_2$ x $2H_2O$, 50 mg $l^{-1}ZnCl_2$, 50 mg $l^{-1}H_3BO_3$, 90 mg l^{-1} $(NH_4)_6$ Mo_7 O_{24} x $4H_2O$, 100 mg $l^{-1}Na_2SeO_3$ x $5H_2O$, 50 mg l^{-1} $NiCl_2$ x $6H_2O$, 1 g l^{-1}EDTA,1 ml l^{-1} 36% HCl, 0.5 g l^{-1} resazurin, 1 l H_2O]. Then the flasks were shaken well and the pH measured. After closure of the flasks with the OxiTop measuring head, they were incubated in a shaking incubator at 25°C for 1 week. OUR (mg O_2 kg^{-1} dry substrate per day) was calculated as:

$$OUR = MO_2 \, x(V_t - V_l) \, x \, \Delta po_2 / (R \, x \, T_m \, x \, m)$$

where MO_2 is molecular weight of O_2 (= 32,000 mg mol^{-1}), Vt the bottle volume (ml), V_l the sample volume (ml) ΔpO_2 the change in partial O_2 pressure (mbar), R the gas constant (= 83.1441 mbar $mol^{-1}k^{-1}$), Tm the temperature (= 303.14 K), and m the dry weight of the sample (kg).

All soil physicochemical and biological analyses were performed in duplicate. The physico-chemical characteristics and microbial activity of the three organic amendments and unamended topsoil are shown in Table 1.

Disease assessment. Disease severity and incidence were assessed at weekly intervals until 56 days post-inoculation. Severity was evaluated based on a 0 - 4 disease scale (Swanson *et al.*, 2005) where 0 = no symptoms of wilting, 1 = 0 to 25% of plants showing wilting, 2 = 26 to 50% of plants showing wilting, 3 = 51 to 75% of plants showing wilting and 4 = 76 to 100% showing wilting. Disease incidence was calculated as % diseased plants over the total number of plants. For statistical analysis disease severity grades were converted to percentage severity index (PSI) using the formula:

$$PIS = S_{nr} \, x \, 100/N_{pr} \, xM_{sc}$$

where S_{nr} is the sum of numerical ratings, N_{pr} is the number of plants rated and M_{sc} is the maximum score on the scale.

In addition, the area under disease progress curve (AUDPC) was calculated from percentage of disease incidence and severity according to the midpoint rule (Garrett and Mundt, 2000) as:

$$AUDPC = \sum_{i=1}^{n-1} [0.5(x_i + x_{i+1})][t_{i+1} - t_i]$$

where x_i is the percentage of disease severity or incidence at i^{th} assessment, t_i the time of the i^{th} assessment in days from the first assessment date and n is the total number of days disease was assessed. Since severity or incidence (x) was expressed in percent and time (t) in days, AUDPC was expressed in %-days (Campbell and Madden, 1990).

Table 1. Physicochemical and biological characteristics of cocopeat, farmyard manure (FYM) compost, green compost and topsoil before mixing.

Parameters	Cocopeat	FYM	Green compost	Topsoil
%OM	87.5 a [1]	39.4 c	47.6 b	13.6 d
PH ($CaCl_2$)	4.6 d	7.7 a	7.3 b	7.1 c
EC ($uScm^{-1}$)	580.0 c	3334.0 a	2964.0 b	280.0 d
C (g kg^{-1})	488.6 a	208.3 c	259.4 b	42.3 d
N(g kg^{-1})	6.4 c	17.7 b	25.4 a	4.1 c
C/N	77.3 a	11.8 b	10.2 b	10.4 b
DOC (mg kg^{-1})	2980.0 a	1374.0 b	2794.0 a	127.0 c
Nts (mg kg^{-1})	169.5 c	308.8 b	1560.7 a	59.4 d
DON (mg kg^{-1})	44.8 c	72.8 b	107.2 a	4.8 d
NO_3-N (mg kg^{-1})	4.7 d	221.2 b	1434.0 a	50.1 c
NH_4-N (mg kg^{-1})	119.9 a	14.9 c	19.4 b	4.5 d
OUR [2]	30.3 b	38.2 ab	63.3 a	12.5 b

[1] Means with the same letters in the row were not statistically significant ($P < 5\%$). Two replications per determination.
[2] OUR (oxygen uptake rate) was determined as mg O_2 kg^{-1} dry substrate per day.

Statistical analysis. For each response, the validity of model assumptions was verified by examining the residuals as outlined in Montgomery (2008). For some response variables log transformation was needed to meet the normal distribution of the error terms assumptions. Accordingly, Log transformed [log (CFU+1)] CFUs in rhizosphere soil and in plant tissue extracts were analyzed using Proc GLM of SAS v.9 (SAS Institute, USA). Two phases of statistical analysis were employed. The first one involved determining whether the main and/or interaction effects of the fixed factors of interest were significant. The second one involved further analysis to compare the LS means of the treatment combinations of significant interactions. AUDPC was computed for those treatment combinations that had received bacterial inoculation. In order to avoid the influence of unequal replications, AUDPC was analyzed in two phases, first as a 3 x 3 factorial in 7 blocks with the exclusion of control treatment. Hence, the three levels of amendment (cocopeat, FYM compost, green compost) and rate at three levels (1, 5, 10%) were used as fixed factors of interest in the model. When the interaction effect of the fixed factors (amendment and rate) was significant ($P < 0.05$), AUDPC was analyzed by one-way ANOVA using MINTAB V 15 by including the control treatment. In addition, Chi-square ($\chi 2$) tests were conducted on contingency tables with two categories of wilt incidence (wilted or not wilted) for different amendments using SPSS V 15 (SPSS Inc., USA).

Physico-chmical and biological characteristics of amendments, mixes and unamended top soil and yield-associated agronomic response variables were subjected to ANOVA using MINTAB V 15. A least square (LS) means comparison was conducted to separate the means of significant fixed effects. Correlation analyses were carried out using SPSS V 15. To confirm that the observed disease symptom was due to *R. solanacearum* race 3 biovar 2, random re-isolation samples from diseased plants were plated on modified SMSA media and the colony characteristics were noted. At the end, the same colony characteristics were observed. Moreover, latent infection was tested by plating measured and surface-sterilized stems cut from the crown area and ten-fold serial dilutions were plated (50 µl per plate) on SMSA media and the recovered *R. solanacearum* race 3 biovar 2 colonies were counted after 6 days of incubation at 28°C. The data generated were subjected to ANOVA analysis using Proc GLM of SAS v.9 (SAS Institute, USA).

RESULTS

Effects of organic soil amendments on survival of *R. solancearum*. The population densities of *R. solanacearum* in the rhizosphere soil were assessed one week and two months after inoculation, and in extracts from the stem bases two months post-inoculation. There was no interaction between types and rates of amendments one week after inoculation (Fig. 1). However, a marginally significant ($P = 0.08$) difference was observed between the main effects of amendment. A significant ($P = 0.035$) interaction between amendment types and rates was observed in the rhizosphere soil two months after inoculation (Fig. 1). The number of culturable bacteria recovered from rhizosphere soil two months after planting was significantly lower (*ca*. 0.25 x 10^2) in the soil amended with 10% FYM compost compared to control treatment (*ca.* 2.5 x 10^3). Also the bacterial population recovered from soil amended with 10% FYM two months post-inoculation was reduced by 98% compared to the population recovered from soil amended with same amendment and rate one week after inoculation.

Two months after inoculation the numbers of bacteria in plant tissue (stem base) was checked by plating bacterial suspensions extracted from 1 cm stem base on SMSA media. The culturable bacteria recovered from plants grown on amended and unamended soils showed significant interaction between type of amendment and rate (Fig. 2). Latent infection was evident in all treatment combinations except in 10% FYM and 1% green compost amended soils.

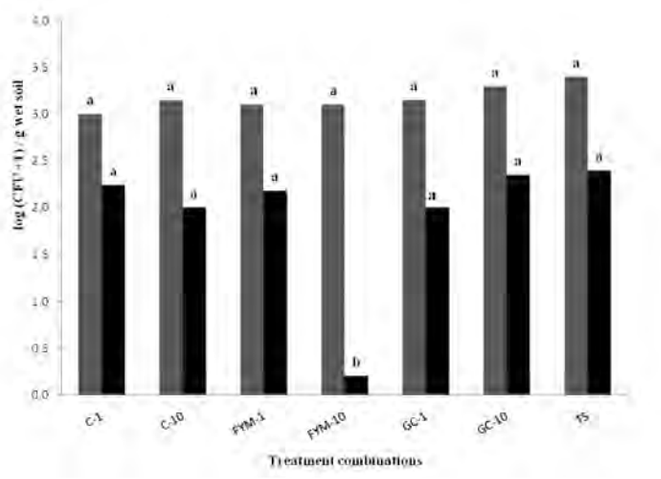

Fig. 1. Culturable population of *Ralstonia solanacearum* race 3 biovar 2 in rhizosphere soil one week (grey bar) and two months (black bar) after inoculation in soils to which different types and rates of organic amendment [1% cocopeat (C-1), 10 % cocopeat (C-10), 1% FYM (FYM-1), 10% FYM (FYM-10), 1% green compost (GC-1) and 10% green compost (GC-10)] were added. Top soil infested with *R. solancearum* (TS) was used as a positive control. Interaction effect between amendment and rate was not significant at ($\alpha = 0.05$) one week post-inoculation whereas a significant interaction was observed two months post-inoculation. Two-week old tomato seedlings were transplanted into pots experimentally infested with 75 ml of *R. solanacearum* suspension at 10^8 CFU ml^{-1} (*ca*. 5 x 10^6 CFU g^{-1} dry soil) and distilled water in control treatments and grown in a lath house for two months.

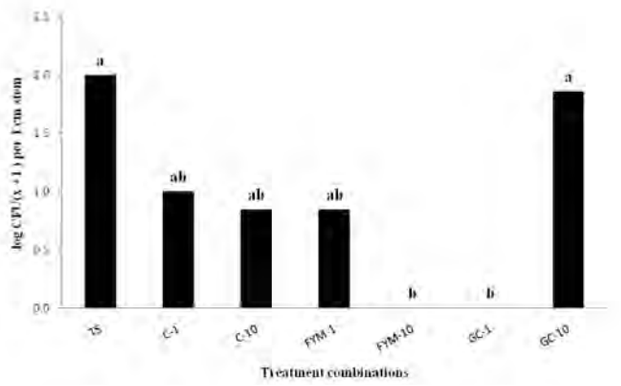

Fig. 2. Culturable population of *Ralstonia solanacearum* race 3 Biovar 2 from the stem base two months after inoculation in soils to which different types and rates of organic amendment [1% cocopeat (C-1), 10% cocopeat (C-10), 1% FYM (FYM-1), 10% FYM (FYM-10), 1% green compost (GC-1) and 10% green compost (GC-10)] were added. Topsoil infested with *R. solancearum* (TS) was used as a positive control. Significant interaction effect between amendment and rate was observed. Two-week old tomato seedlings were transplanted into pots experimentally infested with 75 ml of *R. solanacearum* suspension at 10^8 CFU ml^{-1} (*ca.* 5 x 10^6 CFU g^{-1} dry soil) and distilled water in control treatments and grown in a lath house for two months.

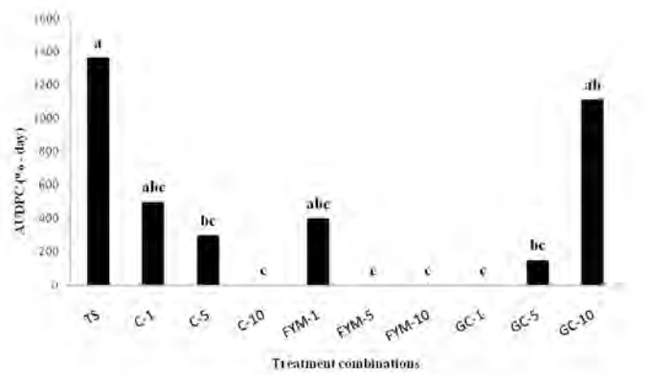

Fig. 3. Bacterial wilt development expressed as AUDPC on tomato plants grown in potting soil treated with different amendments at different rates [1% cocopeat (C-1), 5% cocopeat (C-5), 10% cocopeat (C-10),1% FYM (FYM-1), 5% FYM (FYM-5), 10% FYM (FYM-10), 1% green compost (GC-1), 5% green compost (GC-5) and 10% green compost (GC-10)] or unamended topsoil (TS). Top soil infested with *R. solancearum* (TS) was used as a positive control. Two-week old tomato seedlings were transplanted into pots experimentally infested with 75 ml of a suspension of the pathogen at 10^8 CFU ml^{-1} (*ca.* 5 x 10^6 CFU g^{-1} dry soil) and distilled water in control treatments and grown in a lath house for two months.

Effect of different amendments on disease progress. A significant interaction between type of amendment and amendment rate in terms of AUDPC was evident (Fig. 3). Higher AUDPC was observed in the control treatment compared to topsoil amended with 10%, 5 and 10%, and 1 and 5% cocopeat, FYM and green compost, respectively. Soils amended with 5 and 10% FYM, 10% cocopeat and 1% green compost completely inhibited the effects of *R. solanacearum*, while disease severity reached 43% in the unamended control soil.

As shown by the χ^2-test, more plants were wilted (χ^2 = 16.277, α = 0.05, P = 0.001) in the control treatment than the other soils amended with FYM, cocopeat and green compost (Table 2). There was no significant difference

Table 2. Contingency table for the effect of organic amendments (cocopeat, farmyard manure (FYM), green compost) and topsoil on bacterial wilt incidence among tomato plants 56 days after planting.

Amendment[a]	Wilt classes		Amendment	Wilt classes	
	Wilted	Not wilted		Wilted	Not wilted
Cocopeat	3	39	Cocopeat	3	39
FYM	2	40	FYM	2	40
Green compost	5	37	Green compost	5	37
Control (Topsoil)	6	8			
$\chi 2$ [b]	16.28		$\chi 2$[c]	1.521	
Cocopeat	3	39	FYM	2	40
Control (Topsoil)	6	8	Green compost	5	37
$\chi 2$ [d]	9.929		$\chi 2$	1.403	
FYM	2	40	Green compost	5	37
Control (Topsoil)	6	8	Control(topsoil)	6	8
$\chi 2$	12.44		$\chi 2$	6.37	

[a] Values indicated under the three amendments (Cocopeat, FYM, Green compost) were summed over the three rates.
[b] Significant when $\chi 2 > 7.82$, α = 0.05; $\chi 2 > 11.35$, α = 0.01; $\chi 2 > 16.27$, α = 0.001
[c] Significant when $\chi 2 > 5.99$, α = 0.05
[d] Significant when $\chi 2 > 3.84$ α = 0.05

Table 3. Effects of type and rate of amendment on soil physicochemical and microbial characteristics at the beginning of the experiment. Analysis was performed on duplicate samples and the two replicates were used for ANOVA.

Amendment type and rate[3]	Soil parameters									
	% OM	EC (uScm^{-1})	C (g kg^{-1})	N (g kg^{-1})	C/N	DOC (mg kg^{-1})	Nts (mg kg^{-1})	OUR[1]	NH_4-N (mg kg)	NO_3-N (mg kg^{-1})
C-1	14.1 c[2]	275.0 e	43.5 e	4.1 d	10.6 c	118.2 d	58.7 f	11.4 b	5.2 c	51.0 fg
C- 5	14.9 b	289.0 e	47.7 d	4.1 d	11.5 b	127.1 cd	59.3 e	20.0 b	2.2 e	55.9 e
C-10	16.0 a	448.5 b	54.0 b	4.2 d	13.0 a	129.1 c	60.1 e	14.7 b	5.5 c	51.4 f
FYM-1	13.6 d	326.5 d	43.6 e	4.2 cd	10.4 c	137.6 bc	62.3 e	24.2 b	4.8 c	57.3 de
FYM-5	13.8 d	393.5 c	46.5 d	4.4 c	10.7 c	144.7 b	68.2 d	25.3 b	5.2 c	58.9 d
FYM -10	15.9 a	481.0 a	62.8 a	4.8 a	13.1 a	166.6 a	65.9 d	200.0 a	6.3 b	57.0 e
GC-1	13.7 d	315.0 d	45.9 d	4.4 bc	10.4 c	126.6 cd	71.3 c	13.6 b	6.4 a	64.3 c
GC-5	14.4 c	445.0 b	46.0 d	4.5 b	10.3 c	138.1 bc	88.0 b	27.0 b	5.8 bc	80.3 b
GC-10	14.7 bc	472.5 a	50.0 c	4.8 a	10.4 c	170.9 a	120.2 a	13.2 b	7.1 a	113.3 a
Control[4]	13.6 d	280.5 e	42.3 e	4.1 d	10.4 c	126.7 cd	59.4 e	12.5 b	4.5 cd	50.1 g

[1] OUR (oxygen uptake rate) was determined as mg O_2 kg^{-1} dry substrate per day.
[2] Means followed by the same letters within column of respective parameters are not significant at _ = 0.05.
[3] Type and rate of amendment (1% cocopeat (C-1), 5% cocopeat (C-5), 10% cocopeat (C-10), 1% Farmyard manure (FYM-1), 5 % Farmyard manure (FYM-5), 10% Farmyard manure (FYM-10), 1% green compost (GC-1), 5% green compost (GC-5) and 10% green compost (GC-10)) to topsoil.
[4] Topsoil alone was used as control

($\chi^2 = 1.521$, $\alpha = 0.05$, $P = 0.468$) between green compost, FYM and cocopeat. Pair wise comparison between control and amendments showed highly significant differences in wilt incidence (Table 2). Accordingly, the number of wilted plants in control treatment was significantly higher compared to FYM ($\chi^2 = 12.44$, $\alpha = 0.05$, $P = 0.000$), cocopeat ($\chi^2 = 9.929$, $\alpha = 0.05$, $P = 0.002$) and green compost ($\chi^2 = 6.373$, $\alpha = 0.05$, $P = 0.012$).

Physico-chemical and biological properties of organic matter-amended soil. Amendment by rate interactions were highly significant for all the parameters tested except for pH and DON (Table 3). Soil amended with 10% FYM had higher microbial activity and C content than any other treatment including the non-amended control (Table 3). A significantly higher EC was found in soil amended with 10% FYM or green compost compared to the other amendments. OM and C/N ratio were significantly higher in soil amended with 10% cocopeat and 10% FYM than the other amendments. Soil amended with 10% green compost had a higher NO_3-N and Nts than any of the other amendments. Significantly higher N and DOC contents were also observed in soil amended with 10% green compost compared to soils treated with the other amendments but the difference was not significant between 10% green compost and 10% FYM.

There was a significant negative correlation between per cent disease severity and NH4-N (r = -0.825, P = 0.003). Significant positive correlations occurred between disease incidence (DI) and pH ($CaCl_2$), NO_3-N, DON, and Nts. However, there were significant negative correlations between final DI and EC ($P \leq 0.01$), N ($P \leq 0.05$), and C ($P \leq 0.01$). A significant negative correlation was also found between the number of bacteria recovered from the rhizosphere soil two months after planting and total carbon content (r = -0.865).

Effect of amendment type and rate on shoot and root biomass. The effects of amendment type and rate and inoculation level on yield associated-agronomic parameters are presented in Table 4. In order to assess the interaction effects between amendment, rate and inoculation level, control treatments were excluded from the statistical analysis.

Root fresh and dry weight per pot. For root dry weight (RDW) per pot, F values for amendment ($P \leq 0.001$) and the three-way interaction between amendment, rate and inoculation ($P = 0.03$) were significant, whereas F values for amendment by rate, amendment by inoculation and rate by inoculation interactions were not significant. Application of FYM at 10% rate gave higher root dry weight in inoculated soil than the other two rates (Table 5). No significant effects of different rates of cocopeat and green compost were observed.

Table 4. *P* values of the main and interaction effects of amendment (Am), rate(R) and inoculation (I) on above-ground fresh weight (AGFW), above-ground dry weight (AGDW), root fresh weight (RFW), and root dry weight (RDW).

Fixed effects	AGFW	AGDW	RFW	RDW
Am	*0.001*[a]	*0.001*	*0.001*	*0.001*
R	*0.001*	*0.012*	*0.043*	*0.057*
I	*0.033*	0.340	0.670	0.174
Am x R	*0.023*	0.344	0.956	0.774
Am x I	*0.003*	0.022	*0.001*	0.155
Rx I	0.964	0.749	0.935	0.246
Am x R x I	0.147	0.081	0.386	*0.030*

[a] P values less than 0.001 are shown as 0.001. The p values of significant effects that need further means comparison are shown in italics in the table.

Above-ground fresh and dry weight per pot. The effects of amendment type and rate with and without *R. solanacearum* on above-ground fresh and dry weight are presented in Tables 6 and 7. Accordingly, the three-way interaction between amendment, rate and inoculation for above-ground fresh weight (AGFW) was not significant. However, amendment by inoculation ($P = 0.003$) and amendment by rate interaction ($P = 0.023$), amendment ($P \leq 0.001$), rate ($P \leq 0.001$) and inoculation ($P = 0.033$) were significant. FYM amended to topsoil at 5 and 10% gave the highest AGFW compared to the other amendments (Table 6).

FYM-amended topsoil in the presence of *R. solanacearum* gave the highest AGFW compared to its counterpart without *R. solanacearum*. FYM gave significantly higher AGFW than the other two amendments in inoculated pots (Table 7). For AGDW per pot, the three-way interaction between amendment, rate and inoculation level was marginally significant ($P = 0.081$). Two-way interaction between amendment and inoculation ($P = 0.022$), main effect of rate ($P = 0.012$) and amendment ($P \leq 0.001$) were significant, whereas amendment by rate and inoculation by rate interactions were not significant. Generally, a significantly higher AGDW per pot was observed in FYM-amended soil with *R. solanacearum* (Table 7). FYM-amended soil gave the highest AGDW under inoculated conditions compared to its non-inoculated counterpart. FYM also gave significantly higher AGDW than green compost in non-inoculated pots.

DISCUSSION

Our study revealed an average percentage (*ca.* 81%) reduction of bacterial wilt incidence in amended soils compared to unamended soil. More plants were wilted ($P = 0.001$) in unamended soil than soils amended with FYM, cocopeat and green compost (Table 2) indicating the suppressive effect of the studied amendments on the incidence of *R. solanacearum.* This could be mainly due

Table 5. Interactive effects of amendment types and rates on root dry weight per pot 56 days after planting. Control treatments (positive and negative controls) were excluded from statistical analysis. Values were averaged over the third factor.

Rates	1%		5%		10%	
Inoculation	Not inoculated	Inoculated	Not inoculated	Inoculated	Not inoculated	Inoculated
Amendments						
Cocopeat	1.4 bc	1.2 c[a]	1.5 bc	1.5 bc	1.4 c	1.5 bc
Green compost	1.1 c	1.5 bc	1.4 c	1.6 bc	1.5 bc	1.4 bc
FYM[2]	1.8 ab	1.6 bc	1.7 b	1.8 ab	1.6 bc	2.1 a

[a] Means followed by the same letters were not significant at (P< 5 %).
[b] FYM = farmyard manure.

Table 6. Interacting effects of amendment and rate on above-ground fresh weight per pot 56 days after planting.

Amendments	Amendment rate		
	1%	5%	10%
Cocopeat	56.4 c[a]	60.2 c	61.4 c
Green compost	64.5 c	72.3 bc	75.5 b
FYM[b]	79.2 b	100.2 a	103.1 a

[1]Means followed by the same letters within one parameter were not significant at (P< 5%).
[2] FYM = farmyard manure.

Table 7. Interacting effects of amendment types and inoculation levels on above-ground dry weight (AGDW) per pot 56 days after planting.

Parameters	AGDW	
Inoculation	Not inoculated	Inoculated
Amendments		
Cocopeat	9.1 cd[a]	8.2 d
Green compost	8.9 cd	9.7 c
FYM[b]	11.8 b	13.0 a

[a] Means followed by the same letters within one parameter were not significant at (P< 5%) .
[b] FYM = farmyard manure.

to improvement in soil physicochemical characteristics (OM, EC, and C) and microbial activity of the amended soil to the advantage of crop growth. This was supported by the results of physico-chemical and biological analysis showing that unamended soil was poor in nearly all parameters assessed. Incorporation of composted organic materials increased the availability of essential nutrients needed for rapid growth and development of the plant and non-resident micro-organisms whose populations explode and possibly have negative effects on *Ralstonia*. This enhanced growth may play a significant role in minimizing the damage inflicted on the plant by *R. solanacearum*.

Proper and timely application of nutrients is one mechanism of pest management in agro-ecosystems. Other studies also indicate that soil amendment with compost or manure (Schonfeld *et al*., 2003) or pig slurry (Gorissen *et al*., 2004) reduce populations of soil-borne pathogens including *R. solanacearum*. Messiha *et al.* (2007b) reported a significant reduction in potato bacterial wilt incidence in cow manure-amended soil. Antagonistic microbial population (Schonfeld *et al*., 2003; van Elsas *et al*., 2005), changes in the microbial community (Gorissen *et al*., 2004; Janvier *et al*., 2007) and ammonia toxicity (Kelman, 1953; Michel and Mew, 1998) were suggested as possible mechanisms of suppression. Moreover, timing of compost applications relative to cropping cycles has a significant effect on its biocontrol efficacy (Hoitink and Changa, 2002).

We found that effects of adding organic amendments on disease suppressiveness and survival of the pathogen were different depending on application rate. Significantly higher AUDPC was observed in soil amended with 10% green compost compared to topsoil amended with 1% green compost, 10% cocopeat, 5 and 10% FYM. There was no significant difference between control and soil amended with 10% green compost in terms of AUDPC. Electrical conductivity, total nitrogen, DOC, NO_3-N, NH_4-N and total soluble nitrogen contents were significantly higher in 10% green compost amended soil (Table 3). The presence of higher AUDPC in 10% compared to 1% green compost amended soil could be due to availability of large amounts of NO_3-N and total soluble nitrogen in the former case. Availability of nitrogen sources in excess of crop requirement often enhances growth of plants thereby predisposing them to pests and diseases. This is in agreement with earlier reports that bacterial wilt of tomato increased as N-$N0_3$ increased (Gallegly and Walker, 1949; Walker *et al*., 1954).

Our results also indicated a significant positive correlation between final wilt incidence and NO_3, DON, and Nts contents. This is also congruent with the findings of Messiha *et al.* (2007b), who found a significant positive correlation between NO_3-N content, disease severity and wilt incidence. The lack of disease suppression in soil amended with higher rate of green compost could be attributed to the presence of excess nutrients such as DOC that possibly loosen the competition between resident microorganisms, plants and the pathogen for these resources thereby enhancing the survival of the pathogen. Competition for available resources (Alabouvette *et al.*, 2006), is a general regulator of microorganism population dynamics sharing the same ecological niche and having the same requirements.

Islam and Toyota (2004) reported that compost prepared from tree bark or coffee residues enhanced the growth of the bacterium that causes bacterial wilt of tomato instead of suppressing it. The better survival of the *R. solanacearum* observed in soil amended with the highest rate of green compost (mixture of coffee husk and grass) relative to the other amendments and rates in the current study might substantiate the enhancing role of coffee residue reported by these authors. An earlier report indicated that while NO_3-N can be readily assimilated by both plants and resident microorganisms, microorganisms will tend to utilize NH_4^+ more quickly than plants where carbon-based residues are available (Brady, 1974). The presence of higher amounts of DOC and N-NH_4 together with higher NO_3-N in soil amended with 10 % green compost might have resulted in excess NO_3-N for plant uptake which could possibly enhance plant growth and susceptibility to *R. solanacearum*. A significant negative correlation between NH_4-N and final disease severity was observed, possibly due to production of ammonia which can be toxic to the bacteria. Similarly, Messiha *et al.* (2007b) reported a significant negative correlation between ammonium and AUDPC and disease severity. Other workers also reported a reduction in bacterial wilt severity following application of a high dose of nitrogen to sandy soil with ammonia compounds more effective than nitrates (Kelman, 1953; Michel and Mew, 1998).

Soils amended with 5 and 10% FYM, 10% cocopeat and 1% green compost completely inhibited infection by *R. solanacearum*, while disease severity reached 43% in the unamended control soil. Absence of disease, among other factors, could be attributed to improvement in physico-chemical and biological properties of the amended soils. The higher microbial activity found in soil amended with 10% FYM in our study might have contributed to the suppressive effect of FYM at that rate. Islam and Toyota (2004) also reported that bacterial wilt incidence on tomatoes was suppressed in farm yard manure amended soil when the application rate was higher than 4% with 8% application rate being the most effective in terms of disease suppression and growth of tomato plants. The rapid decline in the *R. solanacearum* populations observed in amended and unamended soil in our experiments may have been due to the high organic matter content which was 13.6% in unamended soil and even higher in amended soils

(Table 3). The number of culturable bacteria recovered from rhizosphere soil two months after planting was significantly lower in 10% FYM-amended soil, and there was no latent infection in soil amended with 10% FYM compost. This might be due to enhanced microbial activity in soil amended with 10% FYM. This was supported by the result of disease assessment. Reduced survival of *R. solanacerum* in soils having a higher OM content was also reported by Van Elsas *et al.* (2000, 2005).

Congruent to our original hypothesis, effects of organic amendments on dry matter content of the tomato plant were realized. FYM amended at 5 and 10% gave the highest AGFW compared to the other amendments. FYM amended soil also gave the highest AGDW in inoculated pots compared to the non-inoculated counterpart and green compost in non-inoculated pots. Root dry weight was significantly higher in soil amended with 10% FYM. The higher RDW at 10% FYM amendment in inoculated pots could be an indication of absence of disease. The contribution of additional nutrient incorporated together with bacterial solution, which was not the case in non-inoculated pots cannot be ruled out. AGDW was positively and significantly correlated with NO_3-N, Nts, N, and K. This was likely due to a direct effect on tomato growth, as bacterial wilt was actually increased at high NO_3-N and Nts levels. A 33% increase in marketable yield of tomato due to compost addition was reported by Abbias *et al.* (2002). The yield increment was rate-dependent with the higher compost rate increasing fruit yield more compared to the unamended control.

Generally, our results indicated that amending topsoil with different types and rates of organic amendment can suppress the survival of the pathogen in the soil, and bacterial wilt severity, with higher amendment rates being the most effective except green compost at 10% rate. However, given the aggressive nature of the bacterial wilt pathogen, the disease severity observed in the non-amended control treatment infested with *R. solanacearum* was relatively low (43%). This low incidence of the disease and the rapid disappearance of the pathogen in some treatments may be attributed to the natural suppressiveness of the soil which had a high organic matter level. It would be worthwhile to explore the relative suppressiveness of different Ethiopian soils to this pathogen and determine the additional benefits of different amendments and rates for the management of *R. solanacearum*, as the suppressive efficacy of amendments is influenced by the indigenous characteristics of the soil (Michel and Mew, 1998).

Based on our results, it is also advisable to look into the proportion of the ingredients of compost mixes like coffee residues that are known to have the potential to significantly increase the DOC and NO_3 in order to enhance the suppressive capacity of the composted product.

In conclusion, application of FYM particularly at higher rates (5 and 10%) were best in suppressing bacterial wilt and survival of *R. solanacearum* in the soil, and increasing the yield of tomato, and would be a good option to be further explored for the different types of Ethiopian soils in the major tomato producing regions. The mechanism(s) of disease suppression however, need to be further investigated.

ACKNOWLEDGEMENTS

The financial support for this project was provided by the Netherlands Fellowship Program (NFP) of the NUFFIC as part of the senior author's M.Sc. study at Wageningen University. We appreciate the advisory and technical help from the team at Jimma University College of Agriculture and Veterinary Medicine, namely, Dhuguma Adugna, Derebew Belew, Diriba Muleta, Yehenew Getachew, Amsalu Nebiyu, Mulatu Wakjira, Desalegn W/ Senbet, Alemseged Amanuel and Daniel Demtew. We are thankful to H.D. Halm of the Biological Farming Systems Group of Wageningen University, the Netherlands, for conducting the soil chemical analyses. The technical assistance of Oscar J. de Vos and Dine Volker is gratefully acknowledged.

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Received August 25, 2009
Accepted November 29, 2009

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