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Effect of a rock dust amendment on disease severity of tomato bacterial wilt

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Abstract Nutrients are important for growth and development of plants and microbes, and they are also important factors in plant disease control. The objective of this study was to evaluate the effect of a rock dust used as a fertilizer in maintaining health of soil and tomato plants under greenhouse conditions. Four treatments—including M (commercial organic fertilizer), A (rock dust soil amendment), M + A (commercial organic fertilizer + rock dust soil amendment) and CK (blank control)—were examined for their effect on soil properties, soil enzymatic activity, plant growth and control efficacy against tomato bacterial wilt. Treatments A and M + A were significantly better than other treatments in changing soil pH, increasing it from acidic (pH 5.13) to nearly neutral (pH 6.81 and 6.70, respectively). Enzymatic activities in soil were notably influenced by the different treatments—particularly treatment M + A, which increased the activities of alkaline phosphatase, urease, catalase and sucrase to a greater extent in soil. There was no significant difference ($P < 0.05$) in the effects of treatments A and M + A on tomato plant height, stem diameter and biomass. The effect of the four treatments on the chlorophyll content and photosynthetic rate (in decreasing order) were M + A, A, M and CK. The replicate greenhouse experiments showed that the control efficacies of treatments M + A, A, and M against bacterial wilt were respectively 89.99, 81.11 and 8.89 % in first experiment and with the efficacies of 84.55, 74.36, and 13.49 % in the replicate; indicating that rock dust played a key role in the plant–soil interaction. The raised soil pH and Ca content were the key factors for the rock dust amendment controlling bacterial wilt under greenhouse conditions.

Keywords Rock dust · *Ralstonia solanacearum* · Soil enzymes · Soil nutrients · Plant health

Introduction

In recent years sustainable agriculture has become one of the most important issues in agriculture. In addition, plant diseases continue to play a major limiting role in agricultural production (Dordas 2008). Soil-borne pathogens of plants cause various diseases in crops, e.g. root and crown rots, vascular wilting, take-all and damping-off. The extent of their harmful effects range from mild symptoms to catastrophes causing destruction of large fields of agricultural crops. Thus, they are major and chronic threats to food production and

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ecosystem stability worldwide. Examples of such soil-borne plant pathogens in the fungal kingdom are *Fusarium oxysporum* and *Rhizoctonia solani*; and in the bacterial kingdom *Ralstonia* (previously named *Pseudomonas*) *solanacearum* (Yabuuchi et al. 1995), which is generally regarded as one of the most severe plant pathogenic bacteria, causing great economic losses worldwide (Hayward 1991).

Management of soil-borne diseases is difficult, especially in continuously cropped soil, even using fungicides and fumigants. Fungicides can affect human health and the environment, and pathogens can develop resistance to them. Methyl bromide is an ozone-depleting chemical, and its use for treating soil was banned under the Montreal protocol by the end of 2004 (Minuto et al. 2006; Omar et al. 2006). The control of plant diseases using classical pesticides raises serious concerns about food safety, environmental quality and pesticide resistance, which have dictated the need for alternative pest management techniques. One alternative to fungicides is biocontrol with beneficial microorganisms (Li et al. 2008; Zhang et al. 2008). In the recent decades, many microorganisms have been tested for their capacity to suppress soil-borne pathogens in agriculture (Slininger et al. 1998). However, microorganisms with biocontrol potential in tests in vitro and/or in bioassays often have inconsistent behavior under field conditions. This has been the major impediment to large-scale use of biocontrol agents in agriculture (Weller et al. 1995).

Plant diseases result when a susceptible host and a disease-causing pathogen meet in a favorable environment. If any one of these three conditions is not met, there is no disease (Fang 1998). Nutrients are important for growth and development of plants, and also pathogens, and are important factors in disease control (Agrios 2005). All the essential nutrients can affect disease severity (Huber and Graham 1999). In particular, nutrients can affect the disease tolerance or resistance of plants to pathogens by affecting their rate of growth and their state of readiness to defend themselves against pathogenic attack (Linus-Muriithi and Irungu 2004). The combined effect of soil physical limitations and nutrient depletion results in reduced crop productivity through direct effects of inadequate nutrition and through declining plant resistance to pathogen infection (Muchovej et al. 1980). Thus, improving the physical and chemical characteristics of soils is one way of reducing the severity of soil-borne diseases, e.g. availability of nutrients, soil pH, nitrogen (N) form and calcium (Ca) level can all play a major role. Adequate levels of Ca can reduce clubroot in crucifer crops (e.g. broccoli, cabbage and turnips). The disease is also inhibited in neutral to slightly alkaline soils (pH 6.7–7.2) (Jones et al. 1989); a direct correlation between adequate Ca levels, and/or higher pH, and decreasing levels of *Fusarium* spp. occurrence has been established for a number of crops, including tomatoes, cotton, melons and several ornamentals (Campbell and Greathead 1990). Also, the nutrient status of the soil and the use of particular fertilizers and amendments can have significant impacts on the pathogen's environment (Sullivan 2004). Nitrate forms of N fertilizer can suppress Fusarium wilt of tomato, while the ammonia form increases disease severity. The nitrate form tends to make the root zone less acidic; however, the beneficial effects of high pH are lost by using acidifying ammonium-N. Tomato studies have shown that use of nitrate–N in soil with an already high pH results in even better wilt control (Ko and Kao 1989).

Despite the importance of nutrients in disease control being recognized for some of the most severe diseases, the correct management of nutrients for control of disease in sustainable agriculture has received little attention (Huber and Graham 1999; Dordas 2008). It is important to manage nutrient availability through fertilizers or changing the soil environment to influence nutrient availability, and so control plant disease in an integrated pest management system (Graham and Webb 1991; Huber and Graham 1999). The objectives of the present study were (1) to investigate the effect of a novel soil amendment and its application on tomato growth, soil properties and disease severity of tomato bacterial wilt, and (2) accordingly to find out the key factors playing a part in the process of plant disease control.

Materials and methods

Bacterial strain and growth condition

Ralstonia solanacearum strain ZJ3721 (Li et al. 2010) was grown for 2–3 days on YPGA (per L: yeast extract 5 g, acto-peptone 5 g, glucose 10 g and agar 15 g) agar or broth at 28 °C.

Soil and fertilizers

The yellow brown soil employed in the greenhouse experiment was collected from a field that suffered from severe wilt diseases with continuous vegetable cultivation of more than 5 years in Qixia District (32°08′N, 118°54′E), Nanjing, Jiangsu Province, China. Two kinds of fertilizers, soil amendment and organic fertilizer were used. The soil amendment was a rock dust which was a mixture from natural products generated by cutting the accessible materials, including quartz, abundant biotite, equivalent proportions of potassium feldspar, plagioclase, olivine, and rice straw at a weight ratio of 1:3:2:1:1:2. It was passed through a 2 mm (20 meshes) sieve prior to use. Organic fertilizer was bought from an organic Agriculture Development Co Ltd (Xinyi, Jiangsu, China). It was made from pig mature and rice straw at a ratio of 4:6 (V/V) to adjust their C/N ratio to about 30:1–35:1. And the compost materials were piled and turned repeatedly with a wheel loader for about 60 days. The chemical properties of soil, soil amendment and fertilizers are shown in Table 1.

Physico-chemical analysis of soils and fertilizers

The physical and chemical properties of organic fertilizer, soil amendment, and original soil for greenhouse experiment were analyzed before the experiment, but other soil samples were done at the end of experiment. The pH was measured in H_2O and in 0.1 M KCl (1:2.5 powder:solution ratio) using a pH meter. Total N was determined by sulphuric acid digestion using Se, $CuSO_4$ and K_2SO_4 as catalyst and determined by the regular Kjeldahl distillation method (Bremner and Mulvaney 1982). Available N was estimated by alkaline permanganate method suggested by Subbiah and Asija (1956). The total P was determined by Vanado-Molybdate phosphoric yellow colorimetric procedure (Jackson 1967). Available P was analyzed by the Olsen method (Olsen et al. 1954). Total K was determined using an atomic absorption spectrophotometer after wet digestion of a 1 g sample with triple acid mixture (10 ml of HNO_3, 4 ml of $HClO_4$, and 1 ml of HCl). Available K was extracted with 0.5 mol l^{-1} NH_4OAc (pH 7.0) and analyzed by flame emission spectrometry. Na, Ca, Mg, Fe, Al, Mn and Zn were extracted with HNO_3, HF and H_3BO_3 and measured using atomic absorption spectrometric methods APHA (American Public Health Association), AWWA (American Water Works Association), WEF (Water Environmental Federation) (1998). B was extracted with 1 mol l^{-1} HCl and measured by curcuma colorimetry (Dible et al. 1954). Other elements were measured by inductively coupled plasma (ICP) method.

Enzyme assay

The same amount of soil for analysis the enzymatic activities were collected from every pot in each replicate (three replicates for each treatment) and mixed together thoroughly, so every treatment produced three mixed samples which were air-dried at room temperature and passed through a 2 mm sieve for enzyme activities analysis. All enzyme activities were assayed in triplicate with one control.

Activities of urease (EC 3.5.1.5), catalase (EC 1.11.1.6), alkaline phosphatase (EC 3.1.3.1), and sucrase (EC 3.2.1.26) were detected according to Guan (1986) with some minor modifications. For analysis of soil urease activity, 5 g soil was taken into 50 ml conical flask, and 10 ml of 10 % urea solution and 20 ml citric acid buffer (pH 6.7) were added into flask. Soil sample was incubated at 37 °C for 24 h. After 24 h, the solution was filtered and 0.5 ml of filtrate was taken into 50 ml vol. flask, and 20 ml distilled water and 4 ml of mixed reagent (Phenol + NaOH) were added. Then, 4 ml of sodium hypo chlorite solution was added, mixed and made the volume to 20 ml with distilled water, and absorbance of color was measured at 578 nm. Catalase activity in

Table 1 The total nutrients in soil amendment and organic fertilizer

Treatments	N (g/kg)	P	K	Ca	Mg	Fe	Mn	Zn	B	Co	Ni	Al	Na
Amendment	11.60	0.98	22.02	8.82	8.76	26.80	0.39	0.32	0.14	0.02	0.03	72.80	13.79
Organic fertilizer	22.40	27.01	27.37	48.65	17.72	8.14	0.92	2.04	0.21	0.004	0.10	20.80	87.57

soils was measured using the $KMnO_4$ titrimetric method. In brief, 3.0 g fresh soil was placed in 100 ml measuring flask and 40 ml distilled water and 5 ml of 0.3 % H_2O_2 solution into flask which was shaken thoroughly for 20 min. 5 ml of 3 N H_2SO_4 was mixed to cease the reaction and the suspension was filtered. The residual H_2O_2 was determined by titration with 0.1 N $KMnO_4$. Soil phosphatase activity was measured by disodium phenyl phosphate method. Briefly, 5 g of soil sample was carefully transferred into 250 ml flask and 2 ml of toluene was added to inhibit the growth of microorganisms. After standing for 15 min, added 20 ml of 0.5 % (w/v) disodium phenyl phosphate prepared in acetic acid buffer (pH 5), and sample was incubated at 37 °C for 24 h. After incubation, 100 ml of 0.3 % $Al_2(SO_4)_3$ solution was added to the sample, filtered, and 3 ml of filtrate was taken into 50 ml vol. flask. Then 5 ml of borate buffer (pH 9.4) and 4 drops of indicator were added, and made up the volume. The absorbance of color in the solution was measured at 660 nm. Sucrose activity was measured by 3,5-dinitrosalicylic acid colorimetry method. Place 3 g of soil (<2 mm) in a 50-ml Erlenmeyer flask, add 0.2 ml of toluene and 5 ml of MUB (Skujins et al. 1962), swirl the flask for a few seconds to mix the contents, add 5 m of the 10 % sucrose solution (final concentration = 145 mM), and swirl the flask again for a few seconds. Then stopper the flask and place it in an incubator at 37 °C for 24 h. To determine the reducing sugars in the resulting soil filtrate, pipette a 1-ml aliquot into a 50-ml test tube. Then treat the reaction mixture with 5 ml of deionized water, 2 ml of 2 M NaOH, and 2 ml of the color reagent. Pass a stream of nitrogen gas through the reaction mixture for 10 min. With inverted 50-ml beakers on top of each test tube, place all of the tubes into a boiling water bath for 5 min and then allow them to cool to room temperature. Measure the color intensity with a spectrophotometer at a wavelength of 540 nm.

Determination of chlorophyll in tomato leaves

For measurement of chlorophyll content, the procedure for plant leaves sampling like collection the soil for enzymatic activities assay. Each treatment included three mixed plant samples and analyzed in triplicates. Leaf tissue for chlorophyll measurement was harvested using a cork borer that yields 0.5 cm diameter leaf discs that are 0.20 cm^2 in area; excised discs were weighed, enabling the chlorophyll data to be expressed in relation to fresh weight.

Chlorophyll content was assayed as described by Porra et al. (1989). Briefly, chlorophyll was extracted from 50 mg of tomato leaf tissue in 1 ml of 80 % (v/v) acetone containing 2.5 mM sodium phosphate buffer (pH 7.8) on ice. After centrifugation at 4 °C for 20 min at 16,000×*g*, absorbance at 663.6, 646.6 and 750 nm was measured with a spectrophotometer (DU800, Beckman, USA).

Photosynthetic rate assay

Terminal leaflets of three randomly selected, fully developed, young sunlit tomato leaves (approximately 20 days after unfolding) in each treatment were used for photosynthetic rate measurement. A photosynthesis system (LI-6400; LI-COR, Lincoln, NE, USA) with a red/blue LED light source (LI6400-02B) mounted on a 6-cm^2 clamp-on leaf chamber was used to determine the photosynthetic rate—performed in the greenhouse at atmospheric concentration of carbon dioxide at 1,000 μmol m^{-2} s^{-1} of incident photosynthetically active radiation at 25 °C.

Greenhouse experiment

The greenhouse experiments were conducted twice in the year of 2011 and 2012 and each followed the same procedure. Each pot (12 cm in height and 12 cm in diameter) was filled with 1 kg of soil in the greenhouse experiment. Previous experiments established that 10 g of the soil amendment or 10 g of the organic fertilizer were optimal to separately promote the growth of tomato seedlings. There were four treatments: A—soil amendment (each pot received 10 g of soil amendment mixed thoroughly with soil); M—organic fertilizer (each pot received 10 g of organic fertilizer mixed thoroughly with soil); M + A—soil amendment combined with organic fertilizer (each pot received 10 g of soil amendment and 10 g of organic fertilizer mixed thoroughly with soil); and CK—control (no fertilizers).

Tomato seedlings (cv. Shanghai 903) aged 30 days, from a greenhouse maintained at 30 °C and 14/10 h photoperiod, were transplanted to each pot in which the soil was pretreated according to the experiment design. There were three replicates with 36 seedlings

per treatment. Of *R. solanacearum*, 10 ml at a concentration of ca. 1×10^7 CFU/ml was poured on the soil near the roots of the tomato plants after seedlings had grown for 20 days, and disease development was scored daily using a disease severity scale of range 0–4 (0, symptomless plants; 1, 1–25 % of leaves wilted; 2, 26–50 % of leaves wilted; 3, 51–75 % of leaves wilted; and 4, 76–100 % of leaves wilted or dead; Roberts et al. 1988). Disease severity and control efficacy were calculated as follows:

Disease severity = [$\sum$ (The number of diseased plants in this index × disease index)/(total number of plants investigated × the highest disease index)] × 100 %.

Control efficacy = [(Disease severity of control − disease severity of fertilizer-treated group)/disease severity of control] × 100 %.

When the tomato plants had grown for 30 days in pots, the plant height, biomass, photosynthetic rate and chlorophyll concentration of each treatment were measured.

Data analysis

Analysis of variance (ANOVA) for control efficacy, biomass of tomato plant, soil enzymatic activity, photosynthetic rate, and chlorophyll concentration was performed using the SPSS 13.0 (SPSS Inc, Chicago, IL) general linear model (GLM) procedure. Mean comparisons were conducted using ANOVA and a least significant difference (LSD) test ($P < 0.05$). Correlation analysis among disease severity, soil chemical properties, and plant growth parameters was conducted by Pearson's correlation (2- tailed, $P = 0.05$).

Results

Soil properties

Soil chemical parameters were influenced by the different treatments. Before treatment, soil had pH 5.13, which was acidic and unsuitable for plant growth. All treatments increased soil pH compared with CK (with pH 4.81), in particular for treatments A and M + A with pHs of 6.81 and 6.70, respectively. Other treatments did not significantly change pH (Table 2). We also investigated soil macronutrients:

Table 2 chemical characteristics of soils in the greenhouse experiment

Treatments	pH	CEC (mmol/kg)	Total N (g/kg)	Available N (mg/kg)	Total P (g/kg)	Available P (mg/kg)	Total K (g/kg)	Available K (mg/kg)	Available Ca (mg/kg)	Available Mg (mg/kg)	Available S (mg/kg)	Available Mn (mg/kg)	Available Fe (mg/kg)
Original soil	5.13	149.44	1.42	181.21	3.75	41.02	0.90	20.10	2297.2	232.3	66.9	67.0	306.8
M	5.19	156.38	1.45	152.06	3.03	41.19	0.95	20.00	2144.0	244.7	137.0	53.3	294.0
M + A	6.70	153.43	1.33	149.30	2.68	42.97	0.86	20.50	3714.2	306.3	175.1	19.5	113.0
CK	4.81	150.40	1.32	119.36	2.97	37.13	0.85	19.70	2060.8	247.0	71.5	65.2	296.0
A	6.81	150.23	1.27	81.15	4.37	40.07	0.89	19.6	3454.2	266.7	71.8	19.0	119.2

The pH, CEC, and the content of the nutrients were measured after the greenhouse experiment. M denotes commercial organic fertilizer, A denotes soil amendment, M + A denotes commercial organic fertilizer combined with soil amendment, and CK denotes blank control

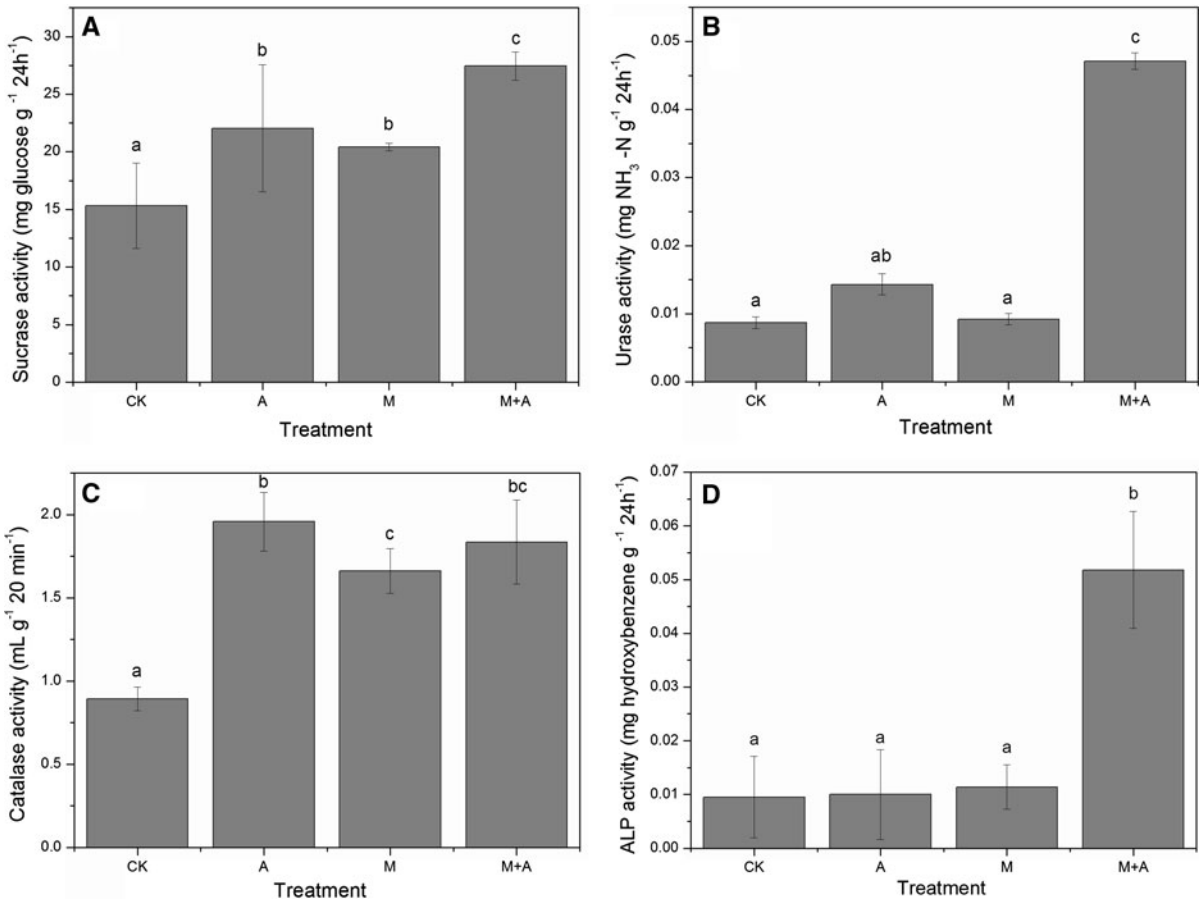

Fig. 1 Effect of different treatments on the soil enzymatic activities. **A** Sucrase, **B** urase, **C** catalase, and **D** alkaline phosphatase. *M* commercial organic fertilizer, *A* soil amendment, *M* + *A* commercial organic fertilizer combined with soil amendment, and *CK* blank control, the ALP indicates alkaline phosphatase

total and available N, phosphorus (P) and potassium (K); medium nutrients: available Ca, magnesium (Mg) and sulfur (S); micro-nutrients: available iron (Fe) and manganese (Mn); and cation exchange capacity (CEC).

Most treatments (except for A) increased soil CEC compared to CK. Treatments M and M + A increased total and available N, P and K concentrations in comparison to CK. Treatment A increased available P and K, but decreased available N compared to CK (Table 2).

The Ca content in M + A and A treatments was 3714.2 and 3454.2 mg/kg, or 80.23 and 67.61 % greater than CK, respectively. These two treatments also increased the Mg concentration in soil. The effect of treatments M + A and A on the contents of Fe and Mn contrasted to Ca and Mg, with lower concentrations of Fe and Mn in treatments M + A and A than in CK (Table 2).

Soil enzymatic activities

Activities of alkaline phosphatase, catalase, sucrase and urease significantly varied among the treatments. The maximum activity of sucrase was 27.45 mg glucose g^{-1} 24 h^{-1} in treatment M + A, and the minimum activity was only 15.34 mg glucose g^{-1} 24 h^{-1} in treatment CK. The activity of sucrase in treatments A and M was 22.00 and 20.42 mg glucose g^{-1} 24 h^{-1}, respectively (Fig. 1A). The trend of urease activity in the different treatments was the same as for sucrase activity (Fig. 1B); the maximum activity being 0.047 mg NH_3 –N g^{-1} 24 h^{-1} in treatment M + A, which was dramatically higher than for other

Table 3 Effect of different treatments on growth of tomato plants

Treatments	Height (cm)	Diameter (cm)	Biomass (g)	Chlorophyll content (mg/g)	Photosynthetic rate ($\mu M\ m^{-2}\ s^{-1}$)
M	33.28 ± 0.89a	0.66 ± 0.15a	1.75 ± 1.01a	2.07 ± 0.03a	5.32 ± 0.69ab
A	49.78 ± 2.82b	0.74 ± 0.08ab	4.24 ± 1.07b	2.18 ± 0.07a	5.88 ± 0.62b
M + A	46.16 ± 3.03b	0.81 ± 0.09b	4.96 ± 1.80b	3.26 ± 0.05b	10.29 ± 0.43c
CK	43.45 ± 1.88b	0.54 ± 0.04c	2.19 ± 0.39a	1.66 ± 0.03c	4.62 ± 0.27ab

The data were expressed as the mean ± standard deviation (SD). Means of three replications followed by the same letter within a column are not significantly different as determined by the LSD test ($P = 0.05$). M denotes commercial organic fertilizer, A denotes soil amendment, M + A denotes commercial organic fertilizer combined with soil amendment, and CK denotes blank control

treatments (Fig. 1B). The maximum catalase activity was in treatment A (1.96 ml g^{-1} 20 min^{-1}), which was a little higher than treatment M + A (1.66 ml g^{-1} 20 min^{-1}; Fig. 1C). The effect of different treatments on the activity of alkaline phosphatase was the same as for sucrase and urease activities; treatment M + A had the maximum soil enzyme activities (Fig. 1D). Thus treatment M + A was the best choice for increasing soil enzyme activity, followed by treatment A (Fig. 1).

Plant growth

Plant height, stem diameter and biomass of different treatments were investigated. Treatment A best promoted plant growth with a height of 49.78 cm, while the height for treatments M + A, M and CK were 46.16, 33.28 and 43.45 cm, respectively. However, plants of treatment A were higher than for treatment M + A, and stem diameter was less than for treatment M + A (which had the greatest diameter of 0.81 cm). Plants for treatments A and M also had larger stem diameters (with 0.74 and 0.66 cm, respectively) than treatment CK. In addition, treatments M + A had optimal effect on plant biomass, with 4.96 g per plant, respectively. Treatment M produced no significant difference in biomass (1.75 g per plant) compared to CK (Table 3). In conclusion, the effect of treatment M + A could more effectively promote plant growth than other treatments.

Plant health

Chlorophyll content and photosynthetic rate are often used to indicate plant health (Guo et al. 2005), so we investigated the two parameters in tomato leaves for the different treatments. Treatment M + A had the maximum chlorophyll content with 3.26 mg/g fresh leaves, which was significantly ($P < 0.05$) higher than other treatments. Compared with treatment CK, treatments M and A had higher chlorophyll contents (2.07 and 2.18 mg/g, respectively; Table 3). The average photosynthetic rate varied with the different treatments. Treatment M + A had a pronounced effect (10.29 $\mu Mm^{-2}\ s^{-1}$) compared to the other treatments: M, A and CK treatments had photosynthetic rates of 5.32, 5.88 and 4.62 $\mu M\ m^{-2}\ s^{-1}$, respectively (Table 3).

Fig. 2 Systemic protection against bacterial wilt in tomato triggered by different treatments. *M* commercial organic fertilizer, *A* soil amendment, *M + A* commercial organic fertilizer combined with soil amendment, and *CK* blank control

Table 4 Efficacy of different treatments for control tomato bacterial wilt

Treatment	First time (2011 summer)		Second time (2012 summer)	
	Disease severity (%)	Control efficacy (%)	Disease severity (%)	Control efficacy (%)
M	68.33 ± 1.44a	8.89 ± 1.92a	60.55 ± 1.84a	13.49 ± 1.70a
A	14.16 ± 3.81b	81.11 ± 5.09b	17.95 ± 1.33b	74.36 ± 1.87b
M + A	7.50 ± 2.50b	89.99 ± 3.34b	10.81 ± 1.93c	84.55 ± 2.77c
CK	75.00 ± 4.33c	–	70.01 ± 1.63d	–

The greenhouse experiment was conducted twice in 2011 and 2012, separately. The data were expressed as the mean ± standard deviation (SD). Means of three replications followed by the same letter within a column are not significantly different as determined by the LSD test ($P = 0.05$). M denotes commercial organic fertilizer, A denotes soil amendment, M + A denotes commercial organic fertilizer combined with soil amendment, and CK denotes blank control

Disease severity of bacterial wilt

The symptoms of bacterial wilt appeared in different treatments in the greenhouse experiments. In 2011, the most severe wilting symptoms were for treatment CK with disease severity of 75.00 %, and treatment M + A had very slight wilting symptoms with disease severity of only 7.50 % (Fig. 2; Table 4). The control efficacies of treatments M + A, A and M were 89.99, 81.11 and 8.89 %, respectively (Table 4). In 2012, the optimal treatment for control bacterial wilt was also M + A treatment with control efficacy of 84.55 %. The control efficacies for treatments A and M were 74.36 and 13.49 %, respectively (Table 4). The two years' results exhibited the similar trend for control the disease, although there were slight differences in the value of control efficacy for different treatments.

Key factor(s) take part in control bacterial wilt

The soil chemical properties, plant growth parameters and disease severity of bacterial wilt in tomato were influenced by different treatments. Correlation analysis was conducted between disease severity and others quantities in order to obtain the key factor(s) in the process of control bacterial wilt in tomato. The result showed that severity of bacterial wilt had significant correlation with soil pH, content of Ca, Mn, and Fe, and tomato biomass with the correlation coefficients of −0.989, −0.998, 0.988, 0.996, and −0.975, respectively (Table 5), which indicates that high soil pH and Ca content is beneficial for plant healthy grow under this greenhouse conditions. However, low content of Fe and Mn will promote plant growth.

Discussion

There is increasing demand for authoritative information on the impact of agricultural land management on soil quality. This information is required to support the development of sustainable crop production practices and improve economic returns to growers (Nelson et al. 2010). Fertilizer use is a key factor for increasing agricultural production and its utilization has increased rapidly in the last four decades, mainly due to adoption of high yielding and nutrient-responsive cultivars in large parts of China. Producing high yields has increased the use of chemical fertilizers, especially in long-term continuously cropped fields, leading to excesses in macronutrients, but deficiencies of medium- and micro-nutrients in soil; accordingly, the balance of nutrients in soil has been disrupted, causing a series of problems under these conditions (Parr and Papendick 1983). In the present study, a novel soil amendment—composed of many kinds of mineral nutrients (macro-, medium- and micro-nutrients; Table 1) and necessary for plant growth and soil health—was evaluated for its effects as a fertilizer on soil properties, plant growth and severity of tomato bacterial wilt.

Soil pH measures acidity or alkalinity, which determines the nutrient availability to plants. The tomato plants in treatments M + A and A were healthy compared with other treatments (Fig. 2), largely attributed to the increases of original soil pH from acidic (pH 5.13) to neutral, because some nutrients become 'tied up' in the soil at certain pH levels. For example, acid soils can lead to deficiencies of P, Ca, Mg and molybdenum, as well as toxic levels

Table 5 Pearson correlation analysis between disease severity and soil and plant parameters

	Total N	Total P	Total K	pH	Mn	S	Biomass	Photosynthetic rate	Height
r	0.561	−0.332	0.270	− 0.989*	0.988*	0.319	−0.975*	0.761	0.717
	Available N	Available P	Available K	Ca	Fe	Mg	Diameter	Chlorophyll content	
r	0.260	0.642	−0.377	− 0.998*	0.996*	0.859	0.918	0.787	

r Indicates correlation coefficient

* Correlation is significant at the 0.05 level (2-tailed)

of Mn and Al. Alkaline soils can have deficiencies in Fe, Mn, B, Cu and Zn (Badawy et al. 2002; Wang et al. 2006; Du Laing et al. 2008). In addition, nutrient deficiencies weaken plants and make them more vulnerable to pests and diseases. However, strong and healthy plants that receive sufficient water, nutrition and sunlight, will build up a natural resistance to pests and diseases. Thus, changing soil pH to neutral is one reason for the soil amendment maintaining tomato health.

Balance of the nutrient budget in soil–plant systems is important in maintaining crop yield and soil fertility during long-term cultivation (Dang 2005). In general, the greatest benefit to plants is when full nutrient sufficiency is provided (Datnoff et al. 2006; Huber and Haneklaus 2007). Elad et al. (2010) found that soil-applied biochar induced systemic resistance to the foliar fungal pathogens *Botrytis cinerea* (gray mold) and *Leveillula taurica* (powdery mildew) on pepper and tomato. The soil amendment used in the present study included many kinds of mineral nutrients necessary and beneficial to plant growth. The parameters of tomato height, stem diameter, biomass, chlorophyll content and photosynthetic rate in treatment A were greater than for treatments CK and M. Additionally, the disease severity in treatment A was also significantly lower compared with CK. This indicates that, as well as the changed soil pH, another reason for the function of the soil amendment in disease control might be the supply of adequate and balanced mineral nutrients which induced plant resistance and/or weakened pathogen virulence—as nutrients can affect disease development by affecting plant physiology or pathogens, or both (Marschner 1995; Dordas 2008).

Ca is an important nutrient affecting the susceptibility of plants to diseases, and the stability and function of plant membranes. Ca deficiency induces membrane leakage of low-molecular-weight compounds (e.g. sugars and amino acids) from the cytoplasm to the apoplast, which stimulates infection by pathogens (Marschner 1995). Adequate soil Ca is needed to protect peanut pods from infections by *Rhizoctonia* and *Pythium* spp. and application of Ca to soil eliminates these diseases (Huber 1980). Ca confers resistance against *Pythium*, *Sclerotinia*, *Botrytis* and *Fusarium* spp. (Graham 1983). The concentration of Ca in soil of treatments M + A and A was higher than for other treatments, and may influence

their control efficacy against bacterial wilt in tomato plants.

Mn has an important role in lignin biosynthesis, phenol biosynthesis, photosynthesis and several other functions (Graham and Webb 1991; Marschner 1995). Although Mn application can affect disease resistance, the use of Mn is limited, due to the ineffectiveness and poor residual effect of Mn fertilizers on most soils that need Mn supplements and also the complex soil biochemistry of Mn (Dordas 2008). Fe is an essential element for bacteria due to its participation in the tricarboxylic acid cycle, electron transport, amino acid and pyrimidine biosynthesis, DNA synthesis and other critical functions (Earhart 1996). When the Fe^{3+} concentration was >300 μM, the expression of the virulence gene was enhanced significantly after 10 h by addition of Fe into a culture for the growth of *P.s. syringae* (Kim et al. 2009). The content of Mn and Fe were less in treatments M + A and A compared with CK, which are also factors decreasing the disease severity of bacterial wilt in tomato plants and beneficial to plant resistance against pathogens.

Management practices used in agriculture influence soil properties, nutrient use efficiency and crop production. Conventional crop management systems that rely on inorganic fertilizers and agrochemicals have, in recent years, increased agricultural productivity (Pimentel 2005). However, too much chemical fertilizer does not help plants grow and can cause negative environmental effects. Additionally, application of inorganic fertilizers may accelerate decomposition of organic residues and potentially reduce aggregate stability (Mäder et al. 2002). To prevent this problem, farmers can use organic fertilizers or reduce inorganic fertilizer amounts (Stockdale et al. 2000). In the present study, some parameters associated with plant health and soil quality in treatment M + A had more beneficial effects than other treatments, showing that soil amendment used with organic fertilizer could produce more favorable results for plant and soil. For example, plant chlorophyll is abundant in nature, harnessing solar energy to perform various metabolic functions through the process of photosynthesis (Anthony and Michael 2005). Thus plants can benefit from higher chlorophyll levels and become healthier. Higher chlorophyll levels resulted from soil fertilized with the soil amendment and organic fertilizer, which both supplied available nutrients and increased soil fertility. Many studies, mainly in arable soils, have shown that organic fertilizers can increase organic matter content, soil biological activity and potential N mineralization (Mäder et al. 2002; Bittman et al. 2005; Fliessbach et al. 2007; Birkhofer et al. 2008; van Eekeren et al. 2009).

In the present study, we employed a novel soil amendment to explore the effect and correlation of fertilizer on/with soil and plant health. The soil amendment (rock dust) was composed of many kinds of nutrients suitable for plant growth and disease control, and greater beneficial effects were obtained when the soil amendment was used combined with organic fertilizer. We also investigate the key factor(s) in control the bacterial disease, but how these factors influence the severity need to further study.

Supplemental results

In order to eliminate the possibility that pathogen will influence the soil physico-chemical properties and enzymatic activities, we have designed a non-inoculated control in the greenhouse experiment, and analyzed their differences in soil physico-chemical properties and enzymatic activities. The results showed that two treatments produced similar results (supplemental Tables 1, 2).

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