

Resistance of Eggplant (*Solanum melongena* L.) to Verticillium wilt Correlates to Microbial Abundance and Soil Enzyme Activities

Zhou B. L.^{1*}, Chen Z. X.¹, Du L.¹, Ye X. L.¹ and Liu Y.F.²

(1. College of Horticulture, Shenyang Agricultural University, Shenyang, 110866, China.

2. Xinxiang Academy of Agricultural Sciences, Xinxiang, Henan, 453002, China.)

ABSTRACT

Aims: To determine the relationship between microbial abundance and enzyme activities of rhizosphere soil from different resistant eggplant cultivars, and resistance of eggplant to Verticillium wilt.

Study design: The changes of microbial and enzymatic activities of the rhizosphere soil from different resistant eggplants after inoculation of *Verticillium dahliae* were analysed.

Place and Duration of Study: The plants were grown in a plastic greenhouse of the Vegetable Crops Experimental Station, and the laboratory experiments were conducted at the Horticulture College, Shenyang Agricultural University from August to November, 2008.

Methodology: 14 eggplant cultivars were selected and inoculated with *Verticillium dahliae* to screen their resistance against Verticillium wilt, and classified according the final disease index. The quantities of main cultivable microorganisms and some functional bacteria were investigated by the serial dilution method. Activities of oxidoreductase and hydrolase enzymes of rhizosphere soil were determined by spectrophotometry or colorimetric titrations.

Results: The correlation analysis among resistance of eggplant to Verticillium wilt, microorganisms and enzyme activities showed that, the abundance of actinomycetes, the ratios of bacteria to fungi and actinomycetes to fungi, and the activities of catalase, polyphenol oxidase, protease and urease, were significantly positively related with the resistance.

Keywords: Verticillium wilt; disease resistance; rhizosphere soil; microbial abundance; soil enzyme activity

*Corresponding author. Tel.: 86-13804013306. Fax: 86-024-88487143; E. Mail: zblaaa@163.com.

1. INTRODUCTION

Verticillium wilt is a destructive disease in eggplant production, which is mainly caused by the infection of *Verticillium dahliae* through root surface to vascular system (Garibaldi et al, 2005; Wang et al., 2005). *V. dahliae* can survive in soil for more than 6 years, and infect many plant varieties, while chemical fungicides can't effect directly to the infected part have no direct effects on infected plants, so the disease is hard to control all over the world (Pegg and Brady 2002; Ligoixakis et al., 2002; Korolev et al., 2008; Berbegal et al., 2010). Therefore, it is necessary to consider the relationship among-between plant, soil and pathogen synthetically, to manage this disease (Park et al, 1963; Han et al, 2006). Plant can affect the pathogen directly through root exudation, or modulate the soil conditions (such as the quantity of microorganisms and the activities of soil enzymes) indirectly (Bertin, 2003; Brusetti et al, 2004). Antagonistic rhizosphere bacteria, actinomycetes and fungi contribute to the induction of remarkable soil suppressiveness against *V. dahliae* (Marois et al, 1982; Berg et al, 1994; Tjamos et al, 2004). Meanwhile, beneficial rhizosphere microorganisms may increase plant growth and development indirectly, through the biocontrol of phytopathogens in the root zone (Weller, 1988; Chen, et al, 1990; Garbeva et al, 2004) and the enhanced availability of minerals (Davison, 1988; Murty and Ladha, 1988), as well as directly through the production of phytohormones (Patten and Glick, 1996). Soil enzymes are important for improving and maintaining soil fertility to ensure productivity, as they take part in organic matter decomposition and nutrient cycling (Verstraete and Voest, 1977; Sinsabaugh et al, 1991; Bohme et al, 2005; Mandal et al, 2007). Oxidoreductases (such as catalase polyphenol oxidase and peroxidase) act in the oxidation reduction in soil (Wang et al, 2011; Finkenbein et al, 2012), and hydrolases are involved in the N (e.g. protease, urease), C (e.g. cellulase, invertase), P (e.g. acid phosphatase) cycling and decomposition of cellulose, lignin, carbohydrate polymers and other biomacromolecules (Verstraete and Voest, 1977; Prieto et al, 2011; Wang et al, 2011). Several studies show that enzyme activities can be used as early indicators of changes in soil properties and microbial activity (Skujins, 1973; Ajwa et al, 1999; Kandeler et al, 2006; Makoi and Ndakidemi, 2008). All these regulatory actions The regulation of pathogens by the plants are is determined by the resistances of different varieties (Bertin et al, 2003; Wu et al, 2010). But the regulatory mechanisms of plant to soil microorganisms and enzyme activities, and their relationship with plant resistance, are not quite clear now. This paper has taken studied different resistant eggplant cultivars to define the relative soil parameters for disease resistance determination, to provide theoretical basis of soil environment management, and to define the direction for

Comment [MF1]: In references list it is written
Chet

integrated management of *Verticillium* wilt of eggplant.

2. MATERIAL AND METHODS

2.1 Plant material

The research group has selected the main eggplant cultivars grown in North China. According to previous experiments, 14 different eggplant cultivars were selected to assess their resistances to *Verticillium* wilt. They were: Bu Lang (BL for short), Liao Qie 6 (L6), HeiYouliang (HL), Hei Mei (HM), Xin Wujin (XW), Bang Lv (BV), Xi'an Lv (XL), Lv Baoshi (LB), Liao Qie 3 (L3), Liao Qie 5 (L5), Li Yuan (LY), Tianjin Kuai Yuan (TY), *Solanum torvum* and *Solanum tovu*.

2.2 Pathogen

The *V. dahliae* was isolated from tap roots and stems of diseased eggplant plants, inoculated to healthy eggplants and isolated again from newly formed symptoms of *Verticillium* wilt, according to Koch's postulate. The identity of isolate was confirmed on the basis of colony morphology, conidiophore formation, conidial production, and presence of microsclerotia, by the Mycology Laboratory of our the Shenyang Agricultural University. The colonies were grown at 27°C in dark for 20 days on Potato Dextrose Agar (PDA) medium. Then, put the pure culture of *V. dahliae* was put into sterile distilled water in 250 mL triangular flasks and shook-shaken (100 r·min⁻¹) for a night. The liquid culture was filtrated through two layers of sterile gauze, and adjusted to 1×10⁷ spores·mL⁻¹ with sterile distilled water, using hemocytometer.

2.3 Experimental design

The plants were grown in a plastic greenhouse of the Vegetable Crops Experimental Station at Shenyang Agricultural University from August to November, 2008. The eggplant seeds were sterilized with 10 % H₂O₂, and accelerated germination separately according to their germinating time, to make all cultivars at the same growth stage. The nursery substrates were sterilized at 121.6 °C for 1.5 h, trays and other tools were disinfected with KMnO₄ solution. The seedlings were transplanted into plastic pots (13×13 cm) containing farmland soil (in farmland), peat and horse manure (3:2:1), at the two-leafes growth stage. Each plant was injured through the soil surface with a sterile knife, and inoculated with 10 mL of the *V. dahliae* spore (1×10⁷ spores·mL⁻¹) suspension into the injured place, at the 4-leaf growth stage. Each treatment had fifteen plants and was repeated three times. To accelerate the incidence of *Verticillium* wilt, eggplants were cultured under suitable cultivation, and observed daily/weekly? until investigated when the disease appeared.

2.4 Disease assessment

Comment [MF2]: Explain the way you do this.

Comment [MF3]: What do you mean with "suitable cultivation"?

The disease was assessed on leaf symptoms by a wilt index from 0 to 4, according to Emmanouil and Wood (1981) and Xiao et al (1995). Health incidence, disease incidence and disease index were evaluated every 5 days since the first appearance of the typical wilt, using the following calculations:

Health incidence (%) = (Number of Health Plants/Total Number of Plants) × 100 %

Disease incidence (%) = (Number of Infected Plants/Total Number of Plants) × 100 %

Disease Index = $\frac{\sum (\text{Rating number} \times \text{number of plants with the rating})}{(\text{Total number of plants} \times \text{highest rating})} \times 100$

Classification method of resistance type: The eggplant cultivars were classified to different types according to the final DI (Disease Index). Resistant type (R), $DI \leq 15$; moderate resistant type (MR), $15 < DI \leq 30$; tolerant type (T), $30 < DI \leq 50$; moderate susceptible (MS), $50 < DI \leq 70$; susceptible type (S), $DI > 70$.

2.5 Determination of microbial quantityabundance

The eggplant was taken out from pot carefully, and shaken to remove the needless soil, only rhizosphere soil was retained for further determination. For each cultivar, the rhizosphere soil of 3 plants was taken and well mixed. Ten grams of rhizosphere soil was weighted, put into a 250 mL triangular flask containing 100 mL of sterile distilled water, and shaken for 15 min ($100\text{rpm} \cdot \text{min}^{-1}$). Then after 5 min standing, 1 mL of supernatant were decanted into 9 mL of sterile distilled water (10^{-1}), and then diluted the same way to different concentrations (from 10^{-2} to 10^{-9}). The initial soil suspensions were oven dried to calculate the microbial abundance in each sampled soil. Enumeration Count of culturablecultivable bacteria, actinobacteria, fungi, azotobacteria, ammonifying bacteria, nitrifying bacteria and cellulose decomposing bacteria was done with the soil serial dilution method. 200 μL of each dilution were plated in Petri dishes containing selective media, all sterilized at 121°C for 30 min. Beef extract-peptone medium (for bacteria), Gause's synthetic No.1 medium, modified (for actinomycetes), Martin's medium (for fungi), Ashby medium (for azotobacteria) and Peptone ammonification medium (for ammonifying bacteria). The microflora growing on solid media was assessed by plating each serial soil dilution on agar media and counting the number of colony forming units on each plate. All these plates were incubated at 27°C in darkness, for 2 d for bacteria, 10 d for actinomycetes, 3 d for fungi and ammonifying bacteria and 5 d for nitrifying bacteria, after which colonies were counted. The microflora growing on solid media was assessed by plating each serial soil dilution on agar media and counting the number of colony forming units on each plate. Put Nitrifying bacteria and cellulose decomposing bacteria were counted by putting 1 mL of each dilution into test-tubes (with

Comment [MF4]: Why standing after shaking?
All the soil particles and microorganisms will fall in the bottom of the flask.

Comment [MF5]: Could you explain why you dry the soil after it was diluted into water? The interest of this manipulation is not really clear.

containing 5 mL of adapted liquid medium, per tube) containing Stephenson medium, modified (for nitrifying bacteria) and Имшевецкий's modified (for cellulose decomposing bacteria). Enumeration of nitrifying bacteria (using sulfanilic acid and α -naphthylamine as chromogenic agent) and cellulose decomposing bacteria (adding filter paper without cellulose to exam the exist of cellulose decomposing bacteria) grown in liquid medium was done with the method of most probable number (MPN, using the 3-tube MPN Table), after 15 days' incubation at 27 °C in dark. All these mediums were sterilized at 121 °C for 30 min. Each concentration was inoculated on three plates or tubes, which were three replications (X~~u~~ and Zheng, 1986; Yan, 1988; Yin et al, 2009).

2.6 Determination of soil enzyme activities

The soils (from each cultivar) selected above were air-dried for 7 days, passed through a 1 mm screen, and mixed for the determination of enzymatic activities (X~~u~~ and Zheng, 1986; Yan, 1988; Li et al, 2008; Gu et al, 2009; Zhao et al, 2012). The experiments were repeated three times.

The catalase activity was determined by KMnO_4 titration method. Two grams of soil samples wetted with 0.5 mL of methylbenzene, was were incubated for 0.5 h at 4 °C. After that, 40 mL of distilled water and 5 mL of 0.3% H_2O_2 (as substrate) were added, and oscillated-shaken for 30 min ($120 \text{ r} \cdot \text{min}^{-1}$), then added 5 mL of 3 $\text{mol} \cdot \text{L}^{-1}$ H_2SO_4 were added immediately to terminate the reaction. The remaining H_2O_2 was titrated by 0.01 $\text{mol} \cdot \text{L}^{-1}$ KMnO_4 , and the enzyme activity was defined by milliliters of 0.01 $\text{mol} \cdot \text{L}^{-1}$ KMnO_4 per gram of dry soil. Samples without soil were used as background control.

The polyphenol oxidase and peroxidase activities were determined by means of the pyrogallol method, and expressed by the production of galocatechin for 3 h. For determination of polyphenol oxidase activity, 10 mL of 1% pyrogallol and 2 mL of 0.5% H_2O_2 were added to 1 g of soil in a 50 mL volumetric flask, and incubated at 30 °C for 3 h. The optical density of galocatechin extracted by diethyl ether at 430 nm was measured to express the polyphenol oxidase activity. Samples without soil and without reaction substrate were used as background control. The activity of peroxidase was measured the same way without H_2O_2 as substrate.

The protease activity was analyzed by the ninhydrin colorimetric method. For determination of protease activity, 2 g of soil samples was were wetted by 0.5 mL of methylbenzene and incubated for 24 h at 30 °C with 10 mL 1% of gelatin solution. After incubation, 0.5 mL of 0.1 $\text{mol} \cdot \text{L}^{-1}$ H_2SO_4 and 3 mL of 20% Na_2SO_4 were used to precipitate the proteins. The solutions of soil sample were centrifuged for 15 min (4000 rpm-min), then added 1 mL of 2% ninhydrin solution was added. The mixture was extracted by boiled water for the color

Comment [MF6]: Do you mean "sieve"?

development, and diluted to 50 mL, then ~~measured the~~ optical density at 500 nm ~~was measured~~. Samples without soil were used as ~~background control~~. Finally, protease activity was expressed in terms of $\text{NH}_2\text{-N}$ per gram ~~of dry soil~~ for 24 h at 30 °C.

Comment [MF7]: Write it as it was done for cellulase activity measurement

The cellulase activity was measured by the UV spectrophotometry method, and estimated through the production of glucose. For the determination of cellulase activity, 10 g of soil samples ~~was were~~ wetted with 2 mL ~~of~~ methylbenzene, then ~~added~~ 5 mL ~~of~~ acetate buffer (pH 5.5) and 5 mL ~~of~~ 1% carboxyl methyl cellulose (CMC) ~~were added~~. The mixture was incubated at 37 °C for 72 h, then boiled to stop the reaction. ~~Add~~ 0.3 mg ~~of~~ $\text{KAl}(\text{SO}_4)_2 \cdot 12\text{H}_2\text{O}$ ~~was added~~ to precipitate residual cellulose. The filtrate was diluted with distilled water to 50 mL, then 2 mL ~~of~~ diluent and 5 mL ~~of~~ 0.1% anthrone were mixed and boiled for 10 min to develop color.

The color intensity was measured at 551 nm. Blanks were incubated without substrate or soils.

The invertase activity was measured using sucrose as substrate, the soils (10g) ~~was were~~ mixed with 10 mL ~~of~~ 20% sucrose solution and 10 mL ~~of~~ phosphate buffer (pH 5.5) and incubated at 37 °C. After 24 ~~hr~~, the mixture was diluted with distilled water to a final volume of 50 mL. ~~Mixed~~ 20 mL ~~of~~ diluent ~~were mixed~~ with 10 mL ~~of~~ Fehling reagent and 20 mL ~~of~~ distilled water, then ~~added~~ 3 mL ~~of~~ 33% KI and H_2SO_4 (v/v 1:3) solutions ~~were added~~. ~~Used~~ 0.1 $\text{mol} \cdot \text{L}^{-1}$ hyposulphite solution ~~was used~~ to titrate the mixture from ~~bluebule~~ to white, using starch as the indicator. Blanks were incubated without substrate or soils.

The urease activity was determined by the sodium phenate-sodium hypochlorite colorimetric method. As substrate, 10mL ~~of~~ 10% urea solution and 20 mL ~~of~~ citrate buffer (pH 6.7) were added to 10 g of soil samples before being incubated at 37°C for 24 h. After incubation, the soil samples were shaken for 30 min and filtrated. ~~Poured~~ 1 mL ~~of~~ filtrate ~~was poured~~ into a 50 mL volumetric flask, then ~~added~~ 4 mL ~~of~~ sodium phenate and 3 mL ~~of~~ sodium hypochlorite ~~were added~~. After ~~colouration~~ ~~coloration~~, optical density at 578 nm was measured for the extractions within 60 min. Urease activity was expressed in terms of $\text{NH}_4^+\text{-N}$ per 100 grams ~~of dry soil~~ for 24 h. Samples without soil and without reaction substrate were used as ~~background control~~.

The acid phosphatase activity was measured by the disodium phenyl phosphate colorimetric method, and the activity unit was phenol per gram ~~of dry soil~~ in 24 h at 37 °C. A sample of 10 g ~~of~~ soils and 1.5 mL ~~of~~ methylbenzene were mixed, and ~~added~~ 10 mL ~~of~~ disodium phenyl phosphate ($\text{C}_6\text{H}_5\text{PO}_4\text{Na}_2 \cdot 2\text{H}_2\text{O}$) and 10 mL ~~of~~ acetate buffer (pH 5.0, because the pH values of soil samples were less than 7.0) ~~were added~~. After 24 h incubation at 37 °C, 1 mL ~~of~~ filtrate was transferred into a 100 mL volumetric flask, ~~added~~ 5 mL ~~of~~ borate buffer (pH 9.6) and 1 mL ~~of~~ Gibbs reagent ~~were added~~ to develop color. After ~~diluting~~ ~~dilution~~ to 50 mL volume,

the color intensity was measured at 578 nm. Blanks were incubated without substrate or soils.

2.7 Statistical analysis

The data were statistically analyzed with Excel software. Analysis of variance was performed using the Data Processing System software (DPS). The correlation coefficients and Standard Deviation (SD) were calculated by Statistics Package for Social Science software (SPSS).

3. RESULTS

3.1 Resistance to Verticillium wilt

According to the final disease index (Table 1), *S. torvum* and *S. tovu* were of the resistant types, the disease incidence and resistant index were significantly lower than other cultivars. LY was the only moderate resistant (MR) type cultivar, with the a final disease index of 28.75. XL was of the susceptible type (S), and the only cultivar with a disease incidence of 100%. The remaining cultivars were categorized as tolerant (T) and moderate susceptible types (MS), with the disease index ranging from 35.00 to 58.75.

Comment [MF8]: Indicate the statistical value

Table 1. Resistance of different eggplant cultivars to Verticillium wilt.

Cultivar	2008-10-17			2008-10-22			2008-10-27			Resistant types
	Health	Disease	Disease index	Health	Disease	Disease index	Health	Disease	Disease index	
	incidence	incidence		incidence	incidence		incidence	incidence		
	%	%		%	%		%	%		
BL	95.00	5.00	5.00	50.00	50.00	23.75	35.00	65.00	46.25	T
L6	65.00	35.00	35.00	30.00	70.00	41.25	15.00	85.00	57.50	MS
HL	95.00	5.00	5.00	65.00	35.00	18.75	50.00	50.00	38.75	T
HM	80.00	20.00	17.50	50.00	50.00	31.25	15.00	85.00	57.50	MS
XW	95.00	5.00	5.00	45.00	55.00	33.25	25.00	75.00	53.75	MS
BV	80.00	20.00	12.50	40.00	60.00	41.75	20.00	80.00	55.00	MS
XL	35.00	65.00	45.00	5.00	95.00	48.75	0.00	100.00	71.75	S
LB	95.00	5.00	5.00	65.00	35.00	16.25	40.00	60.00	36.25	T
L3	75.00	25.00	25.00	50.00	50.00	38.25	40.00	60.00	35.00	T
L5	55.00	45.00	30.00	15.00	65.00	40.00	25.00	75.00	58.75	MS
LY	100.00	0.00	0.00	85.00	15.00	15.00	50.00	50.00	28.75	MR

Comment [MF9]: For an easier comprehension, the cultivars could be ranged according their resistant types

Comment [MF10]: Why is it highlighted?

Correlation between resistance and soil factors

TY	90.00	10.00	10.00	35.00	65.00	48.00	35.00	65.00	50.00	T
<i>S. torvum</i>	100.00	0.00	0.00	100.00	0.00	0.00	90.00	10.00	10.00	R
<i>S. torvu</i>	100.00	0.00	0.00	95.00	5.00	5.00	85.00	15.00	12.50	R

Note: The "Resistant types" were classified according the final "Disease index".

BL- Bu Lang; L6- Liao Qie 6; HL -HeiYouliang; HM- Hei Mei; XW- Xin Wujin; BV -Bang Lv ; XL-Xi'an Lv;

LB-Lv Baoshi; L3-Liao Qie 3; L5-Liao Qie 5; LY- Li Yuan; TY -Tianjin Kuai Yuan.

R —resistant type; MR—moderate resistant type; T—tolerant type; MS— moderate susceptible;

S— susceptible type. The same below.

3.2 Relationship between microflora abundance and resistance to *Verticillium* wilt

The abundance of microorganisms was higher in rhizosphere soils than in non-planting soil (CK). The quantities-abundance of bacteria and actinomycetes in rhizosphere soils of resistant cultivars were generally higher than in rhizosphere soil of other types, while the quantities-abundance of fungi in soils of resistant types were lower than the average level (about 1.28×10^6 cfu·g⁻¹) (Fig.1). The disease index of different resistant cultivars was significantly negatively correlated with the amount of actinomycetes, but not correlated with the quantities of bacteria and fungi (Table 2). Meanwhile, the disease incidence was significantly negatively correlated with the abundance of bacteria and actinomycetes, but not correlated with the abundance of fungi. The ratios of B/F (ratio of bacteria to Fungi) and A/F (ratio of Actinomycetes to Fungi) of resistant and moderate resistant cultivars were significantly higher than others. B/F and A/F were significantly negatively correlated with the disease index, with the correlation coefficients of -0.560 and -0.576.

The abundance of functional bacteria showed differences among cultivars, but were uncorrelated with disease incidence and disease index.

3.3 Relationship between soil enzyme activities and resistance to *Verticillium* wilt

As Table3 and Table 4 showed, the enzyme activities in rhizosphere soil were higher than in non-planting soil. Meanwhile, the enzyme activities mainly increased with the improvement of resistance. But the significant differences were only showed among few cultivars, which had greater difference in resistance levels, such as *S. torvum* (R), LY (MR) and XL (S). The enzyme activities of *S. torvum* and LY were extremely significant or significantly higher than XL.

The results of Table 5 showed that, activities of catalase, polyphenol oxidase, protease and urease were

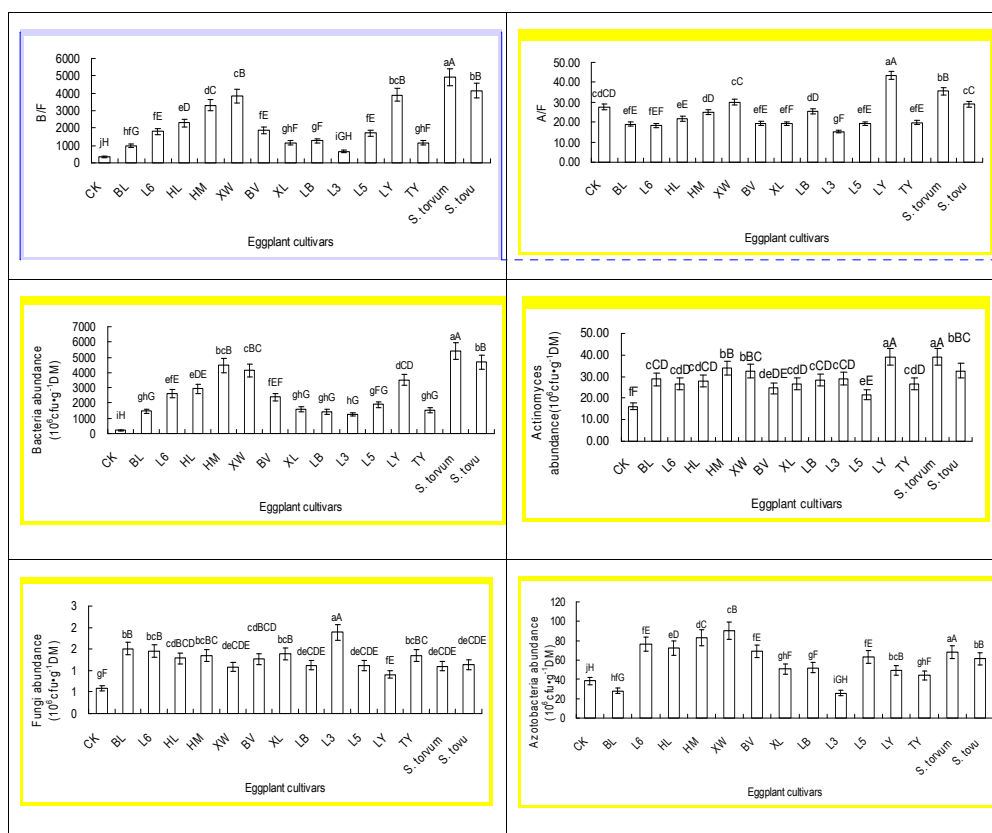
Comment [MF11]: Indicate the statistical value

Comment [MF12]: Statistical value

Comment [MF13]: Statistical value

significantly negatively correlated with disease incidence (with the correlation coefficients of -0.702, -0.559, -0.654 and -0.568 respectively) and disease index (the correlation coefficients were -0.650, -0.601, -0.593 and -0.546 respectively). Other enzyme activities showed no significant correlation with disease incidence and disease index.

Comment [MF14]: For an easier comprehension, the cultivars could be ranged according to their resistant types



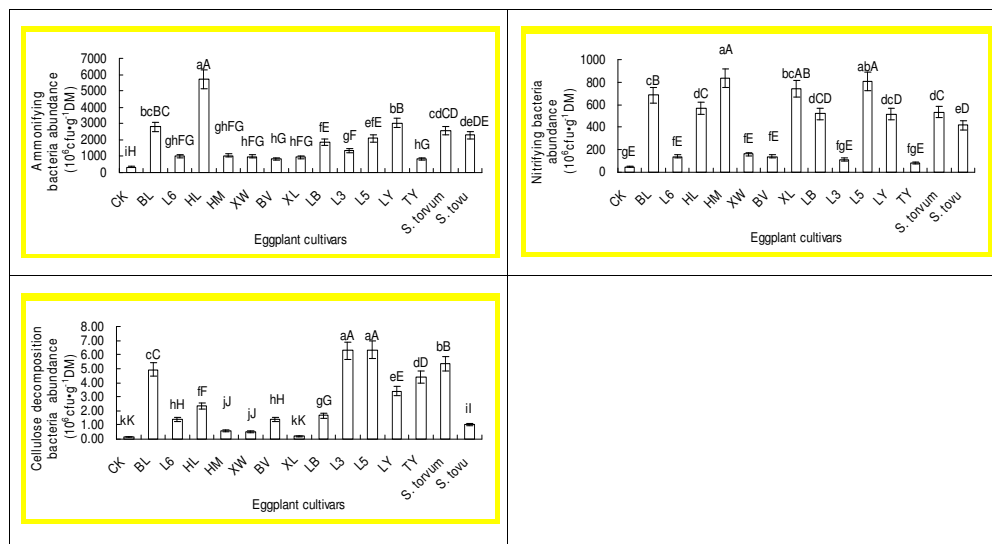


Fig. 1 Comparison of microbial abundance in rhizosphere soil of different resistant eggplant cultivars.

Comment [MF15]: For each graph indicate clearly what is shown, especially then there is an abbreviation.

Note: Different small and capital letters mean significant differences from control at 0.05 and 0.01 levels respectively.

CK: non-planting soil. The unit of microbial abundance is $10^6 \text{ cfu g}^{-1} \text{ DM}$.

Table 2 The correlations between disease incidence, and disease index of eggplant to Verticillium wilt and microbial abundance in rhizosphere soil of different resistant eggplant cultivars.

Correlation coefficients	B/F	A/F	Bacteria (B)	Actinomycetes (A)	Fungi (F)	Azotobacteria	Ammonifying bacteria	Nitrifying bacteria	Cellulose decomposition bacteria
Disease incidence	-0.591*	-0.543*	-0.541*	-0.586*	0.315	0.093	-0.493	0.033	-0.321
Disease index	-0.562*	-0.579*	-0.504	-0.653*	0.261	0.157	-0.449	0.084	-0.294

Note: Correlation coefficients- The correlation coefficients between final disease incidence, disease index (2008-10-27) and rhizosphere microorganisms of the rhizosphere.

* Significant at 0.05 probability level.

Table 3 Variation analyses of Oxidoreductases activity in rhizosphere soil of different eggplant cultivars.

Cultivar	Catalase ($0.01 \text{ mol} \cdot \text{L}^{-1} \text{ KMnO}_4 \cdot \text{mL} \cdot \text{g}^{-1}$)	Polyphenol oxidase ($\text{mg} \cdot 100 \text{ g}^{-1} \cdot 3 \text{ h}^{-1}$)	Peroxidase ($\text{mg} \cdot \text{g}^{-1} \cdot 3 \text{ h}^{-1}$)
CK	1.38 (0.012) iL	39.09 (7.753) fF	0.96 (0.141) hH
BL	1.82 (0.012) iHJ	80.76 (7.283) bcABC	1.89 (0.200) efgEFG
L6	1.67 (0.012) kK	67.80 (3.699) deCDE	2.37 (0.185) deCDE

HL	1.92 (0.000) gFG	71.87 (2.546) cdBCDE	1.19 (0.200) hGH
HM	2.05 (0.024) eDE	84.83 (3.254) abAB	2.89 (0.071) cdBC
XW	1.99 (0.012) fEF	80.01 (5.936) bcABC	3.22 (0.027) bcB
BV	2.09 (0.024) dD	79.64 (6.959) bcABC	1.18 (0.043) hGH
XL	1.87 (0.012) ghGH	68.35 (5.797) deCDE	2.10 (0.228) efDEF
LB	1.80 (0.000) iIJ	75.94 (4.852) bcdBC	2.04 (0.184) hGH
L3	1.75 (0.074) jJ	75.57 (5.602) bcdBC	1.49 (0.063) ghFGH
L5	1.89 (0.012) ghG	59.28 (5.249) eE	1.77 (0.020) fgEFGH
LY	2.21 (0.023) cC	73.35 (0.848) cdBCD	3.52 (0.181) bAB
TY	1.86 (0.012) hGHI	60.95 (6.117) eDE	2.76 (0.181) cdBCD
<i>S. torvum</i>	2.47 (0.360) aA	91.30 (5.298) aA	4.04 (0.369) aA
<i>S. tovu</i>	2.34 (0.048) bB	90.38 (1.697) aA	3.25 (0.562) bcB

Note: Different small and capital letters mean significant differences from control at 0.05 and 0.01 levels respectively.

CK: non-planting soil. Numbers in parentheses are Standard Deviation (SD). The same below.

Table 4 Variation analyses of hHydrolases activity in rhizosphere soil of different eggplant cultivars.

Cultivar	Protease (NH ₂ -Nmg·g ⁻¹ ·24h ⁻¹)	Cellulase (mg·g ⁻¹ ·72h ⁻¹)	Urease (NH ₄ ⁺ -Nmg·100g ⁻¹ ·24h ⁻¹)	Invertase (glucose mg·g ⁻¹ ·24 h ⁻¹)	Phosphatase (phenol g·g ⁻¹ ·24h ⁻¹)
CK	0.108 (0.0045) fG	1.29 (0.044) hF	23.87 (2.124) fE	2.03 (0.026) fE	1.59 (0.238) eE
BL	0.197 (0.0078) eEF	1.91 (0.214) fgE	34.07 (0.174) eD	4.56 (0.202) bBC	3.40 (0.255) bcBC
L6	0.190 (0.0131) eF	2.21 (0.150) deCDE	38.81 (0.386) aAB	4.32 (0.113) dC	3.42 (0.225) bcBC
HL	0.202 (0.0030) deCDEF	1.90 (0.115) fgE	35.67 (0.586) cdCD	4.55 (0.090) bBC	3.42 (0.358) bcBC
HM	0.214 (0.0041) cBCD	2.25 (0.072) deCDE	34.79 (0.610) deD	4.35 (0.025) cdC	3.04 (0.255) cdCD
XW	0.212 (0.0045) cdBCDE	2.44 (0.124) cdCD	36.08 (0.872) cdCD	4.95 (0.090) aA	4.03 (0.207) aA
BV	0.201 (0.0062) deCDEF	2.19 (0.105) defCDE	37.23 (1.226) bcBC	4.69 (0.063) bB	3.52 (0.137) bABC
XL	0.195 (0.0053) eF	2.17 (0.098) defCDE	35.41 (0.333) deCD	4.53 (0.014) bcBC	2.83 (0.166) dD
LB	0.198 (0.0078) eDEF	2.11 (0.100) efgDE	36.20 (0.462) cdCD	4.30 (0.152) dC	4.00 (0.336) aA
L3	0.195 (0.0072) eF	2.15 (0.275) efDE	34.83 (0.113) deD	4.53 (0.152) bcBC	3.51 (0.137) bABC
L5	0.217 (0.0017) bcABC	2.08 (0.160) efgDE	35.75 (1.525) cdCD	4.53 (0.115) bcBC	2.71 (0.209) dD
LY	0.215 (0.0078) cBC	3.55 (0.214) aA	38.34 (0.301) abAB	4.64 (0.104) bB	3.71 (0.079) abAB
TY	0.190 (0.0070) eF	1.86 (0.148) gE	37.25 (0.220) bcBC	3.89 (0.029) eD	3.07 (0.121) cdCD
<i>S. torvum</i>	0.232 (0.0043) aA	2.54 (0.091) cBC	39.80 (0.586) aA	4.99 (0.080) aA	3.45 (0.264) bcBC
<i>S. tovu</i>	0.227 (0.0062) abAB	2.86 (0.195) bB	38.98 (0.313) aAB	4.66 (0.142) bB	2.98 (0.129) dCD

Table 5 The cCorrelations between disease incidence, and disease index of eggplant to Verticillium

wilt and enzymatic activities of rhizosphere soil of different resistant eggplant cultivars.

Correlation between resistance and soil factors

Correlation coefficients	Catalase	Polyphenol oxidase	Peroxidase	Protease	Cellulase	Urease	Invertase	Acid phosphatase
Disease incidence	-0.702**	-0.559*	-0.483	-0.654*	-0.419	-0.568*	-0.370	-0.129
Disease index	-0.658*	-0.602*	-0.472	-0.595*	-0.500	-0.549*	-0.358	-0.260

Note: **: Significant at 0.01 probability level; *: Significant at 0.05 probability level. The same below.

3.4 Correlation between soil enzymic activities and microbial abundance

Table 6 showed the relationship between activities of soil enzymes (including oxidoreductases and hydrolases-) and abundance of microorganisms in rhizosphere soil. The activities of catalase, protease and cellulase were significantly positively correlated with the ratios of B/F and A/F, and the quantity abundance of bacteria, actinomycetes and fungi. Peroxidase showed extremely significant correlation with the ratios of B/F and A/F, and abundances of bacteria and actinomycetes, with the correlation coefficients of 0.793, 0.790, 0.738 and 0.807. The ratio of B/F and bacteria abundance were correlated with the activities of catalase, polyphenol oxidase, peroxidase, protease, cellulase, urease, and invertase, but not related to acid phosphatase activity. There was no significant correlation between acid phosphatase activity and the abundance of the rhizosphere microorganisms.

Comment [MF16]: Already mentioned above

Comment [MF17]: Already mentioned above

Table 6 The correlations between soil enzymatic activities and the abundance of rhizosphere microorganisms of different eggplants cultivars.

Correlation coefficients	B/F	A/F	Bacteria (B)	Actinomycetes (A)	Fungi (F)	Azotobacteria	Ammonifying bacteria	Nitrifying bacteria	Cellulose decomposition bacteria
Catalase	0.869**	0.748**	0.821**	0.703**	-0.596*	0.284	0.199	0.160	-0.043
Polyphenol oxidase	0.631*	0.434	0.691**	0.663**	-0.114	0.191	0.084	0.070	-0.223
Peroxidase	0.793**	0.790**	0.738**	0.807**	-0.528	0.259	-0.124	0.030	-0.077
Protease	0.868**	0.689**	0.827**	0.610*	-0.611*	0.382	0.238	0.358	0.072
Cellulase	0.703**	0.858**	0.567*	0.732**	0.567*	0.105	0.087	0.020	-0.128
Urease	0.604*	0.543*	0.546*	0.415	-0.497	0.286	-0.006	-0.341	-0.068

Correlation between resistance and soil factors

Invertase	0.613*	0.466	0.566*	0.447	-0.314	0.302	0.219	0.100	-0.023
Acid	0.149	0.357	0.068	0.346	-0.160	0.085	0.084	-0.428	-0.135
phosphatase									

4. DISCUSSION

Rhizosphere is a microenvironment made up of root, soil and microorganisms (Lambers et al, 2009), could be more accurately defined as the volume of soil influenced by root activity (Hinsinger, 1998). Plants can affect the soil microorganisms and enzyme activities through root exudates (Landi, et al, 2006; Broeckling et al, 2008; Zhou B.L. et al, 2011; Gao et al, 2012). The effects vary with variety-cultivars and are related to resistance of plant (Kong et al, 2008a, 2008b; Bonkowski et al, 2009; Raaijmakers et al, 2009). Since rhizosphere microbial communities are strongly influenced by root exudates (Brant et al., 2006), it has been hypothesised that plants select for beneficial microbial communities in their rhizosphere (Singh et al., 2007). Furthermore, plants may also play an important role in determining soil enzyme activities, as a mainly source of extracellular enzymes in soil (Martens et al, 1992; Gramss et al, 1999). But there is no unifying understanding of the correlation between resistance of plants, and soil microorganisms quantity-abundance and enzyme activities (Harper, 1950; Larkin, 1993; Li et al, 2007; Yin et al, 2009; Gu et al, 2009; Zhou et al, 2011). Study about the population of rhizosphere microorganisms of 6 cotton cultivars that have different resistance to *V. dahliae* has showed that, the diversity of populations of rhizosphere fungi and actinomycetes are positively correlated with cotton resistance, but the diversity of rhizosphere bacteria population is not closely correlated with resistance (Li et al, 1998). Our experiment analyzed the changes of rhizosphere microbial quantity-abundance and soil enzyme activity under infection of *V. dahliae*, which was completely different with from Li et al (1998), whose experiment was taken under natural conditions without pathogens. In our test, the soils were sampled when disease incidences among cultivars varied. When the roots of susceptible cultivars were go browned and rotted, the root activities (including the excretive, absorptive and enzymatic activities et al) were decrease sharply, which led to the reduction of regulation and control to rhizosphere microorganisms and soil enzyme activities. Conversely, the-resistant cultivars showed greater defense to prevent damages and maintain to root activity, so the regulation and control to rhizosphere soil remained at a high level. Especially, with the increase of B/F and A/F, and-linked with the improvement of catalase, polyphenol oxidase, protease and urease activities, is correlated to the inhibition or the slowing down of the spread of *V. dahliae* was inhibited or slowed.

In this paper, the relationships between the resistance levels of different eggplant cultivars to Verticillium wilt,

Comment [MF18]: Precise what it means

the rhizosphere microbial population and the soil enzyme activities, were systematically analyzed. The ratios of B/F and A/F, the abundance of actinomyces and the activities of catalase, polyphenol oxidase, protease and urease in rhizosphere soil, showed positive correlation with the resistance level of eggplant to Verticillium wilt, so they could be useful indicators to assess soil health level and manage soil environment.

Further studies are needed on relevant physiological and biological process, to determine the possible composition of root exudates which might be involved in the soil management, and reveal the mechanism of root exudation. And with the progress in molecular biology techniques (Söderberg et al, 2004; Wei et al, 2006; Broeckling et al, 2008;), the isolation and identification of microorganisms should be more accurate to avoid the error caused by using traditional serial dilution microbiological methods (only 1-4 % of the microorganisms in soil could be cultured in medium, Amann et al, 1995).

Formatted: Font: 10 pt

Comment [MF19]: And also cellulose, peroxidase and invertase?

Formatted: Font: 10 pt

Formatted: Font: 10 pt

Formatted: Font: 10 pt

Formatted: Font: 10 pt

ACKNOWLEDGMENT

The research was financially supported by the National Natural Science Foundation of China (31171950).

REFERENCES

- Ajwa, H.A., Dell, C.J. and Rice, C.W. (1999). Changes in enzyme activities and microbial biomass of tallgrass prairie soil as related to burning and nitrogen fertilization. *Soil Biology Biochemistry*, 31, 769-777.
- Amann, R.L., Ludwig, E. and Schleifer, K.H. (1995). Phylogenetic identification and in situ detecting of individual microbial cells without cultivation. *Microbiological Reviews*, 59, 143-169.
- Berbegal, M., Ortega, A., Jiménez-Gasco, M.M., Olivares-García, C., Jiménez-Díaz, R.M. and Armengol, J. (2010). Genetic diversity and host range of *Verticillium dahliae* isolates from artichoke and other vegetable crops in Spain. *Plant Disease*, 94(4), 396-404.
- Berg, G., Knaape, C., Ballin, G. and Seidel, D. (1994). Biological control of *Verticillium dahliae* Kleb. by naturally occurring rhizosphere bacteria. *Journal of Plant Disease Protection*, 29, 249-262.
- Bertin, C., Yang, X.H. and Weston, L.A. (2003). The role of root exudates and allelochemicals in the rhizosphere. *Plant and Soil*, 256, 67-83.
- Bohme, L., Langer, U. and Bohme, F. (2005). Microbial biomass, enzyme activities and microbial community structure in two European long-term field experiment. *Agriculture Ecosystems Environment*, 109, 141-152.
- Bonkowski, M., Villenave, C. and Griffiths, B. (2009). Rhizosphere fauna: the functional and structural diversity

of intimate interactions of soil fauna with plant roots. *Plant Soil*, 321, 213-233.

Brant, J.B., Myrold, D.D. and Sulzman, E.W. (2006). Root controls on soil microbial community structure in forest soils. *Oecologia*, 148, 650-659.

Broeckling, C.D., Broz, A.K., Bergelson, J., Manter, D.K. and Vivanco, J.M. (2008). Root exudates regulate soil fungal community composition and diversity. *Applied and Environmental Microbiology*, 74(33), 738-744.

Bruseti, L., Francia, P., Bertolini, C., Pagliuca, A., Borin, S., Sorlini, C., Bruzzese, A., Sacchi, G., Viti, C., Giovannetti, L., Giuntini, E., Bazzicalupo, M. and Daffonchio, D. (2004). Bacterial communities associated with the rhizosphere of transgenic Bt176 maize (*Zea mays*) and its non transgenic counterpart. *Plant and Soil*, 266, 11-21.

Chet, I., Ordentlich, A., Shapira, R. and Oppenheim, A. (1990). Mechanisms of biocontrol of soilborne plant pathogens by Rhizobacteria. *Plant and Soil*, 129, 85-92.

Davison, J. (1988). Plant beneficial bacteria. *Nature Biotechnology*, 6, 282-286.

Emmanouil, V. and Wood, R.K.S. (1981). Induction of resistance to *Verticillium dahliae* and synthesis of antifungal compounds in tomato, pepper and eggplant by injecting leaves with various substances. *Journal of Phytopathology*, 100(3), 212-225.

Finkenbein P., Kretschmer K., Kuka K., Klotz S. and Heilmeyer H. (2012). Soil enzyme activities as bioindicators for substrate quality in revegetation of a subtropical coal mining dump. *Soil Biology Biochemistry*, DOI: [10.1016/j.sbb.2012.05.011](#)

Gao, Y., Miao, C.Y., Xia, J., Mao, L., Wang, Y.F. and Zhou, P. (2012). Plant diversity reduces the effect of multiple heavy metal pollution on soil enzyme activities and microbial community structure. *Frontiers of Environmental Science and Engineering in China*, 6(2), 213-223.

Garibaldi, A., Minuto, A. and Gullino, M.L. (2005). Verticillium wilt incited by *Verticillium dahliae* in eggplant grafted on *Solanum torvum* in Italy. *Plant Disease*, 89(7), 777.

Garbeva, P., van Veen, J.A.V. and van Elsas, J.D.V. (2004). Microbial diversity in soil: selection of microbial populations by plant and soil type and implications for disease suppressiveness. *Annual Review of Phytopathology*, 42, 243-270.

Gramss, G., Voigt, K.D. and Kirsche, B. (1999). Oxidoreductase enzymes liberated by plant roots and their effects on soil humic material. *Chemosphere*, 38, 1481-1494.

Comment [MF20]: In text it is written Chen et al.

Comment [MF21]: Indicate issue and pages

- Gu, Y., Wang, P. and Kong, C.H. (2009). Urease, invertase, dehydrogenase and polyphenoloxidase activities in paddy soil influenced by allelopathic rice variety. *European Journal of Soil Biology*, 45, 436-441.
- Han, X., Wu, F.Z. and Pan, K. (2006). Review on the relation between the root exudates and soil-spread disease. *Chinese Agricultural Science Bulletin*, 22(2), 316-318. (In Chinese)
- Harper, J.L. (1950). Studies in the resistance of certain varieties of banana to panama disease. Part II. The rhizosphere. *Plant and Soil*, 2(4), 383-394.
- Hinsinger, P. (1998). How do plants acquire mineral nutrients? Chemical processes involved in the rhizosphere. *Advances in Agronomy*, 64, 225-265.
- Kandeler, E., Mosier, A.R., Morgan, J.A., Milchunas, D.G., King, J.Y., Rudolph, S. and Tscherko, D. (2006). Response of soil microbial biomass and enzyme activities to the transient elevation of carbon dioxide in a semi-arid grassland. *Soil Biology Biochemistry*, 38, 2448-2460.
- Kong, C.H., Wang, P., Gu, Y., Xu, X.H. and Wang, M.L. (2008a). Fate and impact on microorganisms of rice allelochemicals in paddy soil. *Journal of Agricultural and Food Chemistry*, 56(13), 5043-5049.
- Kong, C.H., Wang, P., Zhao, H., Xu, X.H. and Zhu, Y.D. (2008b). Impacts of allelochemical exuded from allelopathic rice on soil microbial community. *Soil Biology Biochemistry*, 40(7), 1862-1869.
- Korolev, N., Pérez-Artés, E., Mercado-Blanco, J., Bejarano-Alcázar, J., Rodríguez-Jurado, D., Jiménez-Díaz, R.M., Katan, T. and Katan, J. (2008). Vegetative compatibility of cotton-defoliating *Verticillium dahliae* in Israel and its pathogenicity to various crop plants. *European Journal of Plant Pathology*, 122, 603-617.
- Lambers, H., Mougel, C., Jaillard, B. and Hinsinger, P. (2009). Plant-microbe-soil interactions in the rhizosphere: an evolutionary perspective. *Plant and Soil*, 321(1-2), 83-115.
- Landi, L., Valori, F., Ascher, J., Renella, G., Falchini, L. and Nannipieri, P. (2006). Root exudate effects on the bacterial communities, CO₂ evolution, nitrogen transformations and ATP content of rhizosphere and bulk soils. *Soil Biology Biochemistry*, 38, 509-516.
- Li, H.L., Yuan, H.X., Wang, Y., Cai, J.X., Huang, J.L. and Wang, S.Z. (1998). Study on the relationship between diversity of microbes in rhizosphere and resistance of cotton cultivars to *Verticillium dahliae* I. There population of microbes in cotton rhizosphere and cultivars resistant to *V.dahliae*. *Acta Phytopathologica Sinica*, 28(4), 341-345. (In Chinese)
- Li, Y.P., Zhou, B.L., Li, Z.P., Yin, Y.L., Jiang, L.L. and Fu, Y.W. (2007). Relationships between grafted eggplant's verticillium-resistance and rhizosphere soil biological activity. *Chinese Journal of Ecology*,

26(6), 831-834. (In Chinese)

Li, Z.G., Luo, Y.M. and Teng, Y. (2008). Soil and environmental microbiology research method. Beijing: Science Press. (In Chinese)

Ligoxigakis, E.K., Vakalounakis, D.J. and Thanassouloupoulos, C.C. (2002). Host range of *Verticillium dahliae* in cultivated species in Crete. *Phytoparasitica*, 30(2), 141-146.

Larkin, R.P. (1993). Effect of successive watermelon plantings on *Fusarium oxysporum* and other microorganisms in soils suppressive and conductive to Fusarium wilt of water melon. *Phytopathology*, 83, 1097-1104.

Makoi, J.H.J.R. and Ndademi, P.A. (2008). Selected soil enzymes: examples of their potential roles in the ecosystem. *African Journal Biotechnology*, 7, 181-191.

Mandal, A., Patra, A.K., Singh, D., Swarup, A. and Mastro, R.E. (2007). Effect of long-term application of manure and fertilizer on biological and biochemical activities in soil during crop development stages. *Bioresource Technology*, 98, 3585-3592.

Marois, J.J., Johnson, S.A., Dunn, M.T. and Papavizas, G.C. (1982). Biological control of *Verticillium* wilt of eggplant in the field. *Plant Disease*, 66, 1166-1168.

Martens, D.A., Johanson J.B. and Frankenberg, W.T. (1992). Production and persistence of soil enzymes with repeated addition of organic residues. *Soil Science*, 153(1), 53-61.

Murty, M.G. and Ladha, J.K. (1988). Influence of *Azospirillum* inoculation on the mineral uptake and growth of rice under hydroponic conditions. *Plant Soil*, 108, 281-285.

Park, D. (1963). The ecology of soil-borne fungal disease. *Annual Review of Phytopathology*, 1, 241-248.

Patten, C.L. and Glick, B.R. (1996). Bacterial biosynthesis of indole-3-acetic acid. *Canadian Journal of Microbiology*, 42, 207-220.

Pegg, G.F. and Brady, B.L. (2002). *Verticillium* wilts. CAB International, Oxford, UK.

Prieto, L.H., Bertiller, M.B., Carrera, A.L. and Olivera, N.L. (2011). Soil enzyme and microbial activities in a grazing ecosystem of Patagonian Monte, Argentina. *Geoderma*, 16(3-4), 281-287.

Raaijmakers, J.M., Paulitz, T.C., Steinberg, C., Alabouvette, C. and Moënné-Loccoz, Y. (2009). The rhizosphere: a playground and battlefield for soil borne pathogens and beneficial microorganisms. *Plant Soil*, 321, 341-361.

Singh, B.K., Munro, S., Potts, J.M. and Millard, P. (2007). Influence of grass species and soil type on

- rhizosphere microbial community structure in grassland soils. *Applied Soil Ecology*, 36, 147-155.
- Sinsabaugh, R.L., Antibus, R.K. and Linkins, A.E. (1991). An enzymic approach to the analysis of microbial activity during plant litter decomposition. *Agriculture Ecosystems Environment*, 34, 43-54.
- Skujins, J. (1973). Dehydrogenase: An indicator of biological activities in arid soils. *Bulletin Ecological Research Community*, (Stockholm), 17, 235-241.
- Söderberg, K.H., Probanza, A., Jumpponen, A. and Bååth, E. (2004). The microbial community in the rhizosphere determined by community-level physiological profiles (CLPP) and direct soil- and cfu-PLFA techniques. *Applied Soil Ecology*, 25, 135-145.
- Tjamos, E.C., Tsitsigiannis, D.I., Tjamos, S.E., Antoniou, P. P. and Katinakis, P. (2004). Selection and screening of endorhizosphere bacteria from solarized soils as biocontrol agents against *Verticillium dahliae* of solanaceous hosts. *European Journal of Plant Pathology*, 110, 35-44.
- Verstraete, W. and Voets, J.P. (1977). Soil microbial and biochemical characteristics in relation to soil management and fertility. *Soil Biology and Biochemistry*, 9 (4), 253-258.
- Wang, C.Y., Han, G.M., Jia, Y., Feng, X.G., Guo, P. and Tian, X.J. (2011). Response of litter decomposition and related soil enzyme activities to different forms of nitrogen fertilization in a subtropical forest. *Ecological Research*, 26, 505-513.
- Wang, R.H., Zhou, B.L., Zhang, F.L. and Zhang, Q.F. (2005). Allelopathic effects of root extracts of eggplants on *Verticillium wilt* (*Verticillium dahliae*). *Allelopathy Journal*, 15(1), 75-84.
- Wei, X.D., Zou, H.L., Chu, L.M., Liao, B., Ye, C.M. and Lan, C.Y. (2006). Field released transgenic papaya effect on soil microbial communities and enzyme activities. *Journal of Environmental Sciences*, 18(4), 734-740.
- Weller, D.M. (1988). Biological control of soilborne plant pathogens in the rhizosphere with bacteria. *Annual Review of Phytopathology*, 26, 379-407.
- Wu, F.Z., Liu, B. and Zhou, X.G. (2010). Effects of root exudates of watermelon cultivars differing in resistance to *Fusarium wilt* on the growth and development of *Fusarium oxysporum* f.sp. *niveum*. *Allelopathy Journal*, 25(2), 403-414.
- Xiao, Y.H. and Lin, B.Q. (1995). The identification of eggplant germplasms' resistance to *Verticillium wilt*. *China Vegetables*, (1), 32-33. (In Chinese)
- Xu, G.H. and Zheng, H.Y. (1986). Handbook of soil microbiology analysis method. Beijing: Chinese Agricultural Press. (In Chinese)

Yan, C.S. 1988. The soil fertility research methods. Beijing: Chinese Agricultural Press. (In Chinese)

Yin, Y.L., Zhou, B.L. and Li, Y.P. (2009). Effects of grafting on rhizosphere microorganisms of eggplants. Allelopathy Journal, 23(1), 149-156.

Zhao, D., Li, F., Wang, R.S., Yang, Q.R. and Ni, H.S. (2012). Effect of soil sealing on the microbial biomass, N transformation and related enzyme activities at various depths of soils in urban area of Beijing, China. Journal of Soils Sediments, 12, 519-530.

Zhou, B.L., Chen, Z.X., Du, L., Xie, Y.H., Zhang, Q. and Ye, X.L. (2011). Allelopathy of root exudates from different resistant eggplants to *Verticillium dahliae* and the identification of allelochemicals. African Journal of Biotechnology, 10(42), 8284-8290.

Zhou, X.G., Yu, G.B. and Wu, F.Z. (2011). Effects of intercropping cucumber with onion or garlic on soil enzyme activities, microbial communities and cucumber yield. European Journal of Soil Biology, 47, 279-287.

Comment [MF22]: As there is two references called Zhou et al., 2001. Find a way to distinguish them in the text.

Note: Anonymous Reviewer