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SOIL AMENDMENTS AND WATER MOISTURE ON THE CONTROL OF TOMATO BACTERIAL WILT CAUSED BY *RALSTONIA SOLANACEARUM*

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Abstract

Bacterial wilt is one of the most severe tomato diseases worldwide and *Ralstonia solanacearum* is determined to be the pathogen responsible for its spread. Soil amendments such as calcium cyanamide and herb residues have both been proved to be high potential for tomato bacterial wilt control. Disinfection effect and heat generated during the decomposition processes of calcium cyanamide and herb residues effectively controlled *Ralstonia solanacearum* while rare studies are conducted on their mechanisms. In this paper, calcium cyanamide was applied in combination with herb residues under solar greenhouse condition to evaluate their integrated control effects on tomato bacterial wilt. It promoted rapid temperature increases to above 40°C within 15 days. *R. solanacearum* was decreased to the lowest population (5.38 log cfu/g) at 20 d and no recovery was found after treatment. In addition, the optimal soil moisture content for pathogen control was also discussed. Under soil moisture of 85% field capacity, tomato treated with calcium cyanamide and herb residues had the most favorable growing performance with wilt of 3.6% and wilt control efficiency of 79.8%.

Introduction

Tomato has a large consumption in China as fruit and ingredient in dishes. Due to large nutrients input and vegetable production, continuous cropping obstacle caused by soil salinity, soil pathogens, soil acidification and nutrition accumulation becomes inevitable and more severe in tomato cultivation (Wang *et al.* 2010). Researches have shown that continuous cropping of tomato is harmful to soil ecological balance as well as microbial community diversity (Qu and Wang 2008, French 1994, Simujide *et al.* 2013). *Ralstonia solanacearum* (*R. solanacearum*), the causal organism of tomato bacterial wilt (TBW), is believed to be the main cause of the obstacle in tomato's cultivation (Qu and Wang 2008). Chemical disinfection and bioremediation have been applied for the control of TBW (French 1994). Calcium cyanamide ($CaCN_2$), as the most traditional chemical disinfectant, was reported to have suppressive ability of TBW due to its strong alkalinity. Soil noxious microbes and pest can also be eliminated by heat generated during the hydrolyzate of $CaCN_2$ (Simujide *et al.* 2013). The combination of $CaCN_2$ with organic fertilizer (OF) is an innovative approach for the control of TBW. Organic fertilizer is beneficial for heat disinfection through the effect of fermentation (Lian *et al.* 2014). In addition, as the food source and shelter for soil beneficial microorganisms (BMs), OF is helpful for the restoration of soil microbial communities after treated with $CaCN_2$ (Gorissen *et al.* 2004).

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The objects of this study were to evaluate: (1) As the OF, the enhanced effects of herb residues (HRs) on soil heat generation and disinfection when combined with $CaCN_2$, (2) the enhanced effects of combined HRs and $CaCN_2$ and water moisture on the control of *R. solanacearum*, and the improvement of tomato's yield.

Materials and Methods

The experimental soil was heavy clay and the TBW was severe due to a three-year continuous cropping of tomato. Surface soils (0 - 15 cm) were collected and grounded through 2 mm sieve. Soil samples were biologically analyzed after naturally air-drying. Soil microbial population were measured after culture by plate count method (Behera and Sahani 2003). The basal respiration was determined by gas chromatography method (Wu *et al.* 2009). The measured soil biological characters were as follows: bacteria: 3.6 × 106 cfu/g; fungi: 0.6 × 104 cfu/g ; actinomyces: 1.5 × 105 cfu/g; and basal respiration: 8535 mg CO_2 Kg^{-}/d. $CaCN_2$, containing 55% CaO and 20 N with pH value of 12.4, was grainy with diameter ranging from 0.1 - 2 mm. HRs were consisted of organic matter of 721 g/kg, total N of 24.9 g/kg, total P_2O_5 of 7.8 g/kg and total K_2O of 11.3 g/kg with pH value of 6.89.

The treatments were randomly designed and concluded in Table 1. Each treatment was replicated three times. Amendments were prior fully mixed with surface soil (0 - 30 cm) in solar greenhouse covered with mulch films. Soil water moisture (SWM) was adjusted with an interval of 15% field moisture capacity through irrigation. After one month disinfection, inorganic fertilizer was used to equalize the nutrients of each treatment before planting.

Table 1. Plot experimental design of soils under different amendments and field moisture capacity regime.

	Treatment							
	CK	Soil with amendments						
Amendment	/	$CaCN_2$	Herb residues	CaCN2 + Herb residues				
Soil water moisture (% of field capacity)	100	100	100	55	70	85	100	115

The population of *R. solanacearum* was determined by the real - time PCR procedures (Cao *et al.* 2011). TBW incidence and control efficiency (Li *et al.* 2014) were then determined by observing the wilt symptoms. Tomato's yield was calculated based on average weight of single tomato on inflorescence of each plot.

The data were processed and the averages and the standard deviations were calculated by Microsoft Excel. Duncan's multiple range tests (DMRT) were applied for significant analysis of soil temperature, population of *R. solanacearum*, TBW control effects and tomato's yield ($p < 0.05$).

Results and Discussions

In general, soil temperatures were first increased to the peak at 15 days and then gradually declined until 30 days (Fig. 1) with different amendments and soil water moistures. With soil water moisture (SWM) of 100% field capacity, temperatures of soils with amendments increased more quickly than control soil (CK). Soil amended with $CaCN_2$ plus herb residues (CHR) had the highest temperature of 46.2°C and was a little bit larger than soils amended with $CaCN_2$ or HRs at 15 days. It was indicated that, soil temperature was increased due to the heat generation during the hydrolysis of $CaCN_2$ and fermentation of HRs. The combined application of CaCN2 and HRs had an enhanced effect on the increase of soil temperatures. When amended with CHR, soil temperatures with water moisture of 85% field capacity were higher than the other treatments at each recorded day. The order of soil temperatures at 15 days ranged from SWM was 85 > 100 > 55 >70 > 115% field capacity. The results indicated that, SWM is an determining factor for the hydrolysis of $CaCN_2$ and fermentation of HRs (Simujide *et al.* 2013). $CaCN_2$ can be easily dissolved in water and release more heat with larger input of water. However, as the increase of SWM, microbial metabolisms efficiency is decreased due to less oxygen available for soil microbes in soil. Thus, the fermentation of HRs was restrained and less heat was generated (Gorissen *et al.* 2004). Therefore, optimal SWM for both hydrolysis of $CaCN_2$ and fermentation of HRs was also important for soil heat generation.

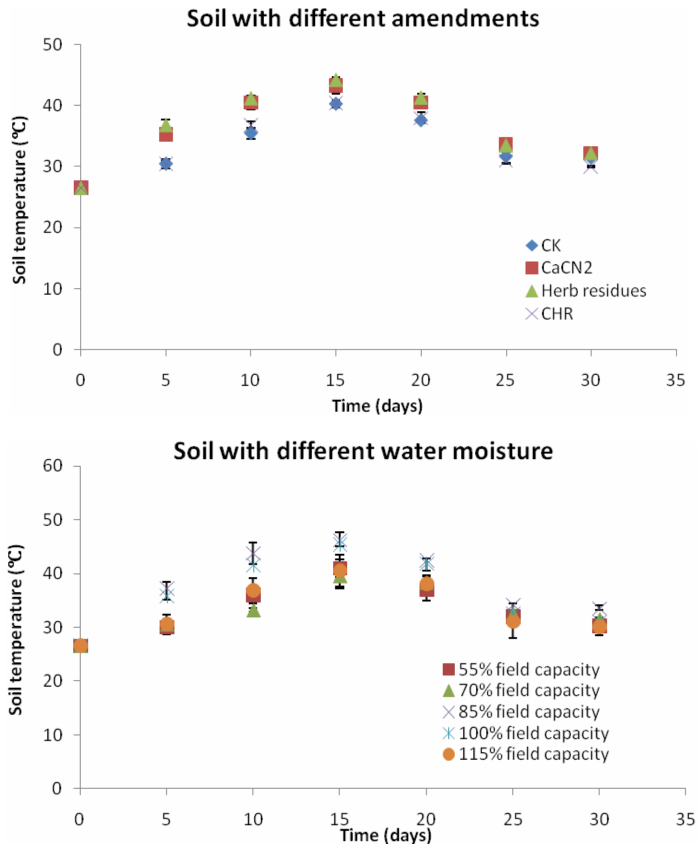

Fig. 1. The effect of different amendments and water moistures on the change of soil temperature.

Soil microbial populations of bacteria, fungi and actinomyces were shown in Figs. 2 - 3. Soil microbes experienced a decline until 15 days and then recovered around to their original levels at 30 days. Processes of pathogens elimination and beneficial microorganisms recovery probably accounted for the trend (Li *et al.* 2011). With water moisture of 100% field capacity, the

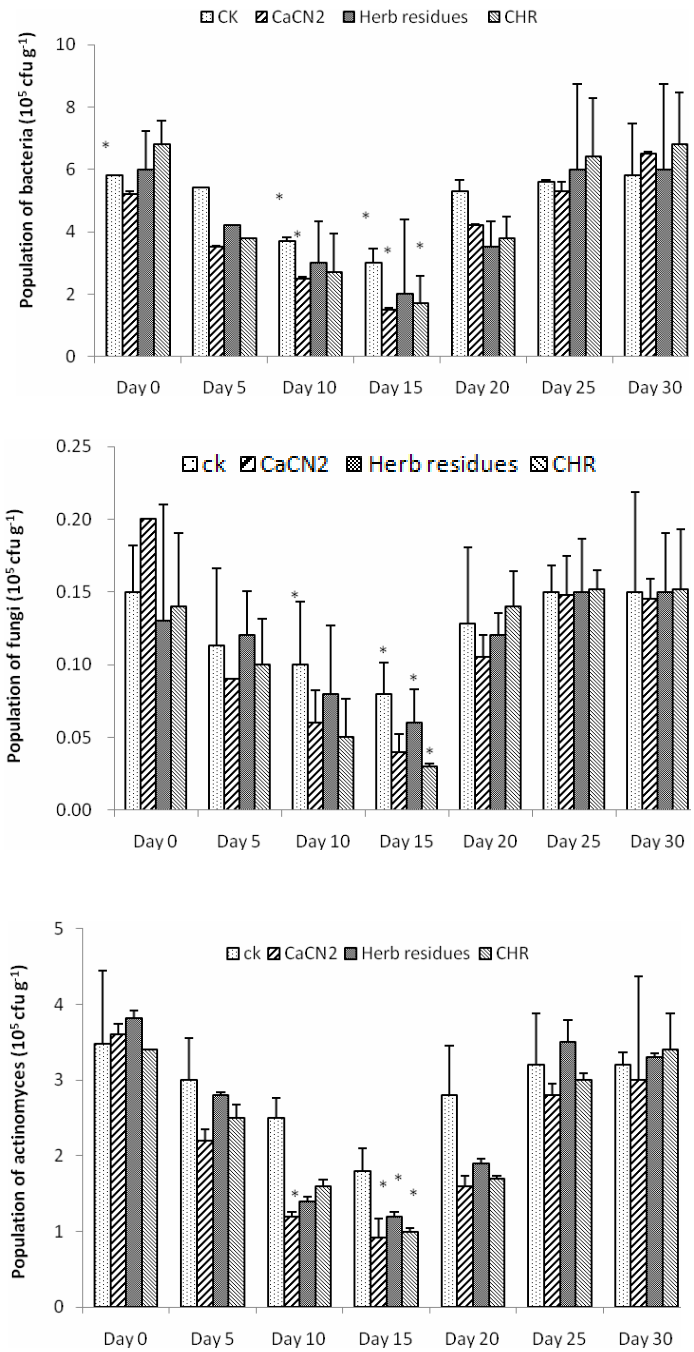

Fig. 2. Microbial population influenced by different amendments.

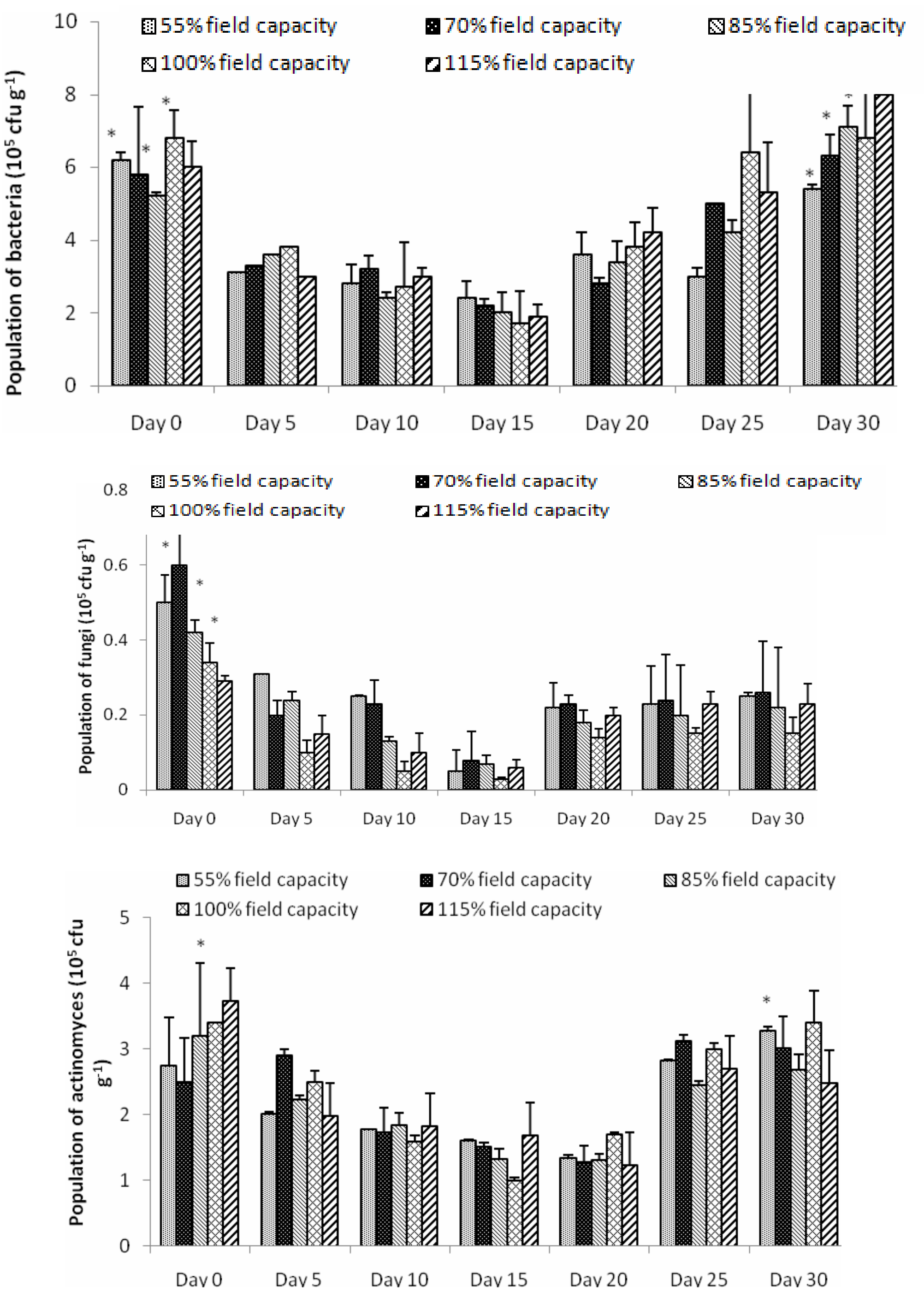

Fig. 3. Microbial population influenced by different water moisture contents.

population of bacteria and actinomyces were mostly decreased by 3.8×10^5 and 2.68×10^5 cfu/g, respectively in soil amended with $CaCN_2$, followed by soil amended with CHR. In addition, the population of fungi was mostly decreased by 0.11×10^5 cfu/g in CHR-amended soil. It was indicated that CHR had good disinfection effect on noxious soil microbes due to the increase of soil temperature. Moreover, CHR-amended soil was most preferred for the recovery of soil beneficial microbes and the population of bacteria, fungi and actinomyces were 6.8 × 105, 0.15 × 105 and 3.4 × 105 cfu/g, respectively at 30 days. Optimal SWM could enhance the disinfection effect of CHR. With soil water moisture of 100% field capacity, populations of bacteria, fungi and actinomyce were mostly decreased by 5.1, 0.31 and 2.4×10^5 cfu/g, respectively at 15 days. Disinfection effects of CHR- amended soils with water moisture of 85% field capacity were also preferable. The results indicated that most noxious soil microbes could be killed with soil temperatures higher than 40°C at 15 days. It had been reported in previous studies that $CaCN_2$ has the suppressive ability on soil-borne diseases in soils planted with tomato and potato (Tremblay *et al.* 2005). It generates high concentration N compounds such as CH_2N_2 and HCN that are detrimental to soil microbes (Scagel 2005). HRs has also been found effective on soil pathogens control (Echeverrigaray *et al.* 2010). It is abundant in nutrients that are beneficial for soil beneficial microbes and, meanwhile, can generate bioactive compounds to restrict pathogens growth (Eyal *et al.* 2011). However, combined use of such amendments on *R. solanacearum*'s control are rare reported.

Table 2. The control of tomato bacteria wilt and tomato's yield influenced by different amendments and water moisture contents.

Amendment	Soil water moisture (%)	Wilt (%)	Wilt in control (%)
CK	100	9.8 ± 1.0	44.9 ± 4.0
$CaCN_2$	100	8.1 ± 1.7	55.1 ± 2.6
Herb residues	100	6.3 ± 1.0	64.4 ± 3.6
	55	10.6 ± 1.3	34.8 ± 3.2
	70	9.8 ± 0.8 *	44.9 ± 4.3
$CaCN_2$ + Herb residues	85	3.6 ± 0.9 *	79.8 ± 2.2 *
	100	4.3 ± 0.7 *	75.8 ± 1.2 *
	115	7.1 ± 0.2	60.1 ± 0.2

*Means were significantly different compared with to at < 0.05.

Populations of *R. solanacearum* influenced with different amendments and soil water moistures were illustrated in Fig. 4. *R. solanacearum's* populations of all the treatments experienced sharp decrease until 15 days and then became steady. With SWM of 100% field capacity, *R. solanacearum's* populations in soils amended with $CaCN_2$, HRs and CHR were 5.44, 5.66 and 5.38 log cfu/g, respectively which were significant smaller than that of CK at 30 days. Population decreasing rate in soil amended with CHR was comparably larger and the smallest population of 5.34 log cfu/g was achieved around 20 days. The results indicated that populations of *R. solanacearum* were related to soil temperatures. $CaCN_2$ and HRs both contributed to heat generation which explained the most favorable disinfection effects in CHR-amended soil with same SWM. In CHR-amended soils, *R. solanacearum* achieved the smallest population (5.03 log cfu/g) at 20 days with soil water moisture of 85% capacity, followed by that of soil (5.34 log cfu/g) with SWM of 100% field capacity. The results indicating that SWM of 85 - 100% field capacity was favorable for disinfection of *R. solanacearum* by CHR. Except for heat disinfection, hydrolysates such as CH_2N_2 and HCN may also restrict the growth of *R. solanacearum*. In addition, HRs, acted as not only nutrients sources but also shelter for soil beneficial microorganisms, promoted microbial immobilization (Bruggen and Semenov 2000). Therefore, development of soil beneficial microorganisms increases their competition for recourses and antibiosis capacity for control of *R. solanacearum*.

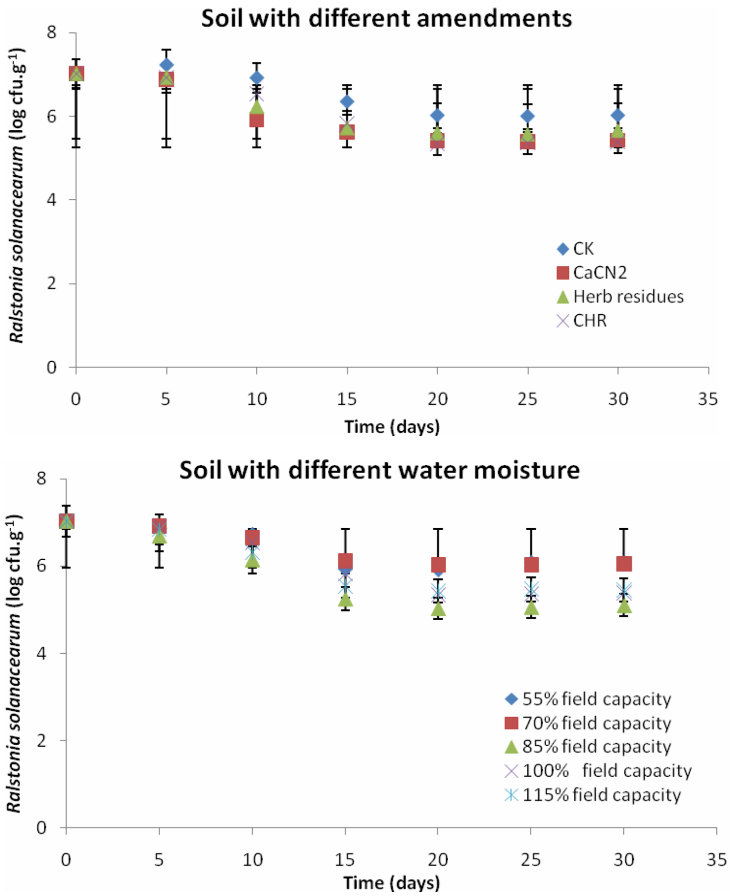

Fig. 4. Population of *R. solanacearum* influenced by different amendments and water moisture contents.

Tomato bacterial wilt (TBW) control effects which included disease incidence and control efficiency were shown in Table. 2.

TBW control effects were most favorable in CHR-amended soils with disease incidence of 4.3% and control efficiency of 75.8%. Followed by $CaCN_2$ and HRs - amended soils with disease incidence of 5.44 and 5.72% and control efficiency of 55.1 and 64.4%, respectively. It was suggested that TBW control effects were significant correlated to *R. solanacearum's* population. Temperatures of CHR- amended soils were rapidly increased to 35 ℃ within 5 days and sustained above 40 ℃ over 10 days. It was found that *R. solanacearum* was more sensitive than other microorganisms in soil and would be killed when the soil temperature was higher than 40 ℃ (Bruggen and Semenov 2000). In terms to SWM, CHR - amended soils with SWM of 85% field capacity had the most favorable disease incidence of 3.6%, control efficiency of 79.8%. Furthermore, CHR - amended soils with SWM of 100% field capacity also had the preferable results. However, dramatic negative effects on TBW control were shown with soil water moistures of 55 or 115% field capacity. The results indicated that, 85% of filed capacity was proved to be the optimal SWM for *R. solanacearum* control. After disinfected with CHR, population of *R. solanacearum* was not recovered to its original level which indicated e preferable disinfection performance of CHR. In addition, extreme soil water moistures had negative effect on *R. solanacearum* control since it affected the amendment decomposition, temperature rising rate and recovery of soil vitality (Klasse 1996).

In conclusion, integrated amendment of CHR improved disinfection effect of soil pathogens and *R. solanacearum*. It had rapid temperature increase and pathogens elimination within 15 days. *R. solanacearum* was decreased to the lowest population at 20 d and no recovery was found after treatment. Soil water moisture was an influencing factor for TBW control and 85% of field moisture capacity was demonstrated to be the optimal SWM. Therefore, soil disinfection using CHR with a period more than 20 days and SWM of 85% field capacity was practical and economical for tomato cultivation.

Acknowledgements

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