Suppression effect of anaerobic digestion effluents on *Ralstonia solanacearum* inoculated in tomatoes (*Solanum lycopersicum* L.)

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Introduction

Tomato plants can be infected by and die from a bacterial wilt of tomato (BWT) caused by a gram-negative bacteria, *Ralstonia solanacearum*. *R. solanacearum* can be found around the world, and destroys tomato plants by preventing the plant from absorbing water. The conventional prevention methods against BWT include applications of wood vinegar (WV) and hot water (HW). However, these methods cannot completely prevent the plant from being infected. Therefore, new prevention methods are eagerly needed to be evaluated. Fertilizer effects of the anaerobic digestion effluents (ADEs) derived from different feedstock on plant growth have been positively evaluated. However, only few studies have examined a suppression effect of ADE application to soils on soil-borne plant diseases.

In this study, a bioassay experiment was performed to compare and evaluate the suppression effect of different ADEs with that of the conventional prevention methods (WV and HW) on tomatoes (*Solanum lycopersicum* L.) infected by *R. solanacearum*.

Materials and Methods

Soil used in this study was Andisol that was sampled in June 19, 2012 (35°68′ N, 139°33′ E). Soil was oven dried (45°C) and sieved (2 mm).

Table 1. Soil chemical characteristics

Soil	T-N (g kg^{-1})	T-P (g kg^{-1})	T-K (g kg^{-1})	pH
Andisol	0.51	0.32	0.58	7.27

Andisol

The experimental treatments were chemical fertilizer only as control (CT), ADEs derived from 2 different feedstock and 2 different pre-treatments (*Egeria densa*: ED; a mixture of cow manure and food waste: CF; ED sterilized: EDS; and CF sterilized: CFS), and conventional methods (WV and HW).

Table 2. Anaerobic digestion effluents chemical characteristics

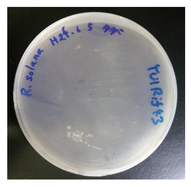
ADEs	T-N (mg L^{-1})	T-P (mg L^{-1})	T-K (mg L^{-1})	T-Ca (mg L^{-1})
ED	1941	465	459	337
CF	1168	84	1083	186
EDS	1884	502	504	379
CFS	1027	75	1212	211

ADEs (CF, ED)

All treatments included chemical fertilizer application necessary for tomatoes (100-160-100 N-P_2O_5-K_2O kg ha^{-1}). *R. solanacearum* was inoculated in the soil after adjusted water content to 50% of water holding capacity. And then, tomato seeds were sowed and tomatoes were cultured for 45 days (Aug. 26–Nov. 4, 2012). During the cultivation period, disease index was analyzed every day. After the harvest, soil pH, colony forming units of soil microbial population (CFU), total soil calcium (T-Ca), soil biomass nitrogen (biomass N), and tomato fresh weight (FW) were analyzed.

Pot study

Diseased tomato (left) and normal tomato (right)

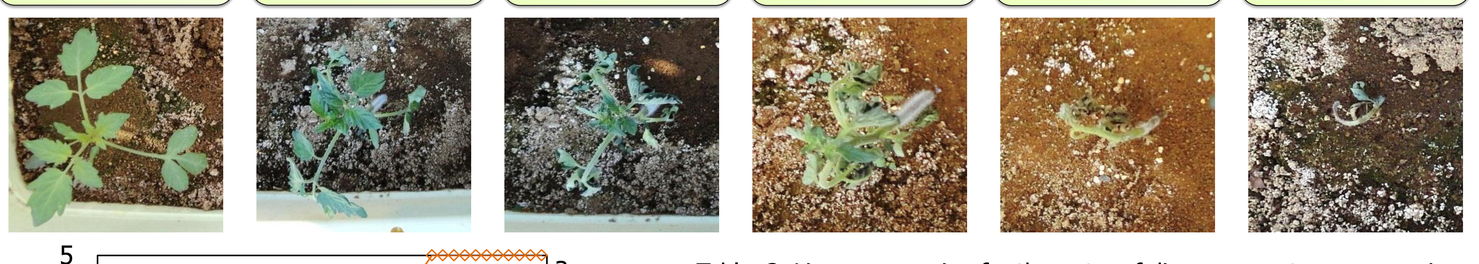


R. solanacearum YUIRif43

Results and Discussion

Disease Index

0: no pathogeny | 1: wilt a few leaves | 2: wilt a half of leaves | 3: wilt all of leaves | 4: wilt the stem | 5: dead



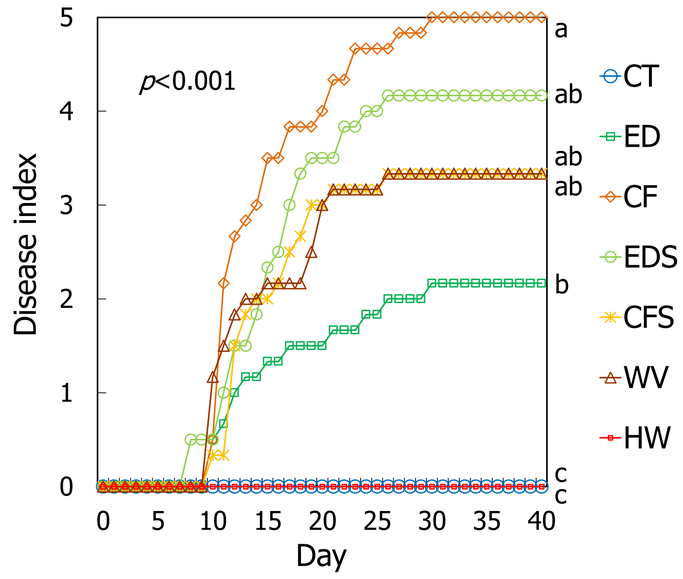


Fig.1. Disease index of BWT by *R. solanacearum* on tomatoes

Table 3. Linear regression for the rate of disease symptom progression

Treatment	Linear regression equation	R^2
ED	y = 0.0779x + 0.6364	0.8705
CF	y = 0.1864x + 1.7016	0.7920
EDS	y = 0.2327x + 0.2310	0.9557
CFS	y = 0.1880x + 0.6520	0.8556
WV	y = 0.1515x + 1.0361	0.8540

- ✓ ED treatment showed lower disease index than other ADE treatments did, but higher disease index compared with HW treatment (**Fig. 1**).
- ✓ ED treatment showed a slower rate of disease symptom progression than other treatments that showed the disease symptom (**Table 3**).
- ➢ **It appeared that ED did not completely prevent but delayed pathogenic process of the BWT.**

Colony Forming Units (CFU)

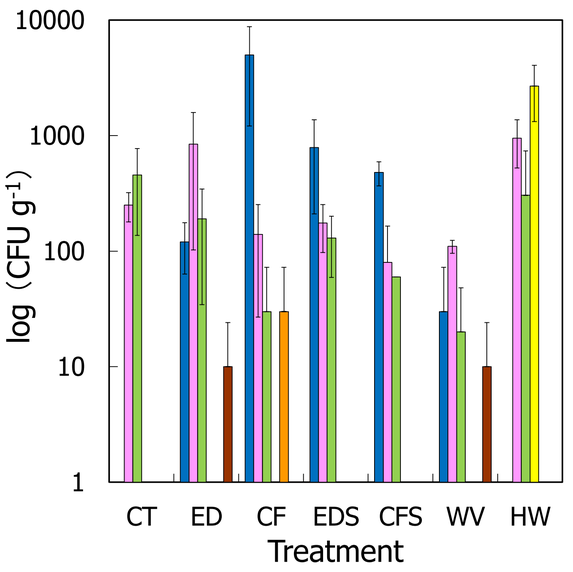


Fig.2. Colony forming units of soil microbial population after the harvest

† All microbes were gram-negative bacteria.

Table 4. Proportion of *R. solanacearum* in soil bacterial population

Treatment	*R. Solanacearum* (%)
CT	0.0
ED	10.3
CF	96.1
EDS	72.1
CFS	77.4
WV	17.6
HW	0.0

- ✓ ED treatment decreased the population of *R. solanacearum* in soil than other ADE treatments, and increased the population of other bacteria (**Fig. 2; Table 4**).
- ➢ **ED increased the population of other bacteria in soil which may have suppressed *R. solanacearum* resulting in the low disease index of the BWT.**

Soil Properties and Plant Growth

Table 5. Soil chemical characteristics and fresh weight (FW) of tomato plants

Treatment	pH	Biomass N (mg kg^{-1})	T-Ca (g kg^{-1})	FW (g)
CT	6.20 (e)	20.4 (bcd)	1.47 (b)	10.1 (a)
ED	6.62 (ab)	17.9 (bcd)	1.89 (a)	7.2 (ab)
CF	6.57 (b)	25.8 (bc)	1.96 (a)	0.1 (b)
EDS	6.64 (a)	3.9 (d)	1.92 (a)	0.3 (b)
CFS	6.34 (d)	7.3 (cd)	1.55 (b)	1.3 (b)
WV	6.43 (c)	58.1 (a)	1.92 (a)	3.6 (ab)
HW	6.41 (c)	29.7 (b)	1.58 (b)	11.2 (a)

- ✓ It has been shown that high soil pH prevents *R. solanacearum* from showing its symptom, but soil pH did not have a correlation with pathogeny of BWT in this study (**Table 5**).
- ✓ WV increased biomass N which was possibly caused by increased fungi tolerant to wood vinegar (**Table 5**).
- ✓ It is know that high T-Ca in soil can prevent *R. solanacearum* from affecting tomatoes, however, in this study, soil T-Ca did not show a correlation with pathogeny of BWT (**Table 5**).
- ✓ Tomatoes exhibited higher FW with the treatments with no pathogeny of BWT, compared with those with the treatments with pathogeny of BWT (**Table 5**).

Conclusions

- ➢ **Disease suppression effect of ADE application on BWT varied depending on its feedstock and pre-treatment (e.g., sterilization), however all ADEs showed higher disease index than conventional methods did.**
- ➢ **ED affected soil microbial population that changed relative abundance of *R. solanacerum* among other gram-negative bacteria.**
- ➢ **ADE derived from *Egeria densa* could not completely prevent but delay pathogenic process of the BWT.**

Future Researches

Mechanism for the disease suppression effect of ADE application to soils should be further investigated in terms of various aspects of the situation such as types of feedstock of ADE, soil microbiota, soil physicochemical properties, cultivation methods and cycles for different crops, and etc.