

# EFFECTS OF SOIL DISINFESTATION TECHNIQUES ON SOIL SUPPRESSIVENESS

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Anaerobicity mediated biological soil disinfestations (ABSDs, Momma et al. 2013) can effectively suppress many soilborne pests and their effects are comparable to conventional chemical fumigation techniques. However, ABSDs have insignificant impact on indigenous soil microorganisms in comparison with chemical fumigation, a significant amount of soil bacteria and fungi survive after ABSDs. These microorganisms are thought to contribute to pathogen suppression through generation of by-product of anaerobic degradation of organic components. And the microbial community is expected to maintain or induce soil suppressiveness. Mazzola et al. (2012) demonstrated that ASD (anaerobic soil disinfestation) treated soil had suppressive nature to apple root infection by *Pythium ultimum*.

In this research, we compared the effects of soil disinfestations on soil suppressiveness to Fusarium wilt of tomato. In 2010, ABSDs using diluted ethanol and wheat bran were performed in a demonstration field in Saitama prefecture in Japan. Ethanol solution (1% v/v, 100 L/m<sup>2</sup>) was applied to the soil for ethanol treatment; wheat bran amendment (1 kg/m<sup>2</sup>) and irrigation (100 L/m<sup>2</sup>) was conducted for wheat bran treatment. The soil surface was covered with a polyethylene film for three weeks. Chloropicrin fumigation was done using a plastic container in laboratory. After soil disinfestations, the treatment soils and non-treated soil were put in plastic containers and bud cells of the pathogen were added and mixed thoroughly (10<sup>5</sup> cells/g dry soil). This means the situation where re-infestation by survived pathogen occurred. Ten tomato seedlings (cv. Regina) were transplanted to the infested soils and grown for 4 weeks. This repeated twice successively with the same soil.

Occurrences of Fusarium wilt were severe in wheat bran and chloropicrin treatment, but were significantly suppressed in ethanol treatment and non-treated control in the first cultivation (Fig. 1). In the second cultivation, severe wilting was observed only in chloropicrin treatment. Because the field was well drained and not able to keep wet condition, wheat bran still remained three weeks after the soil treatment. This might cause significant disease

development in the 1<sup>st</sup> cultivation. Density of soil fungi markedly decreased in chloropicrin treatment and it did not recover even after the 2<sup>nd</sup> cultivation (Table 1). PCR-DGGE revealed the diversity of soil fungi decreased in chloropicrin treatment (Fig. 2).

In 2013, similar test was performed using soil collected from an experimental field of Institute for Horticultural Plant Breeding. Soil (60 kg) put in plastic container was treated with ethanol solution (1%, v/v, 20 L/container), wheat bran and water (200g and 20L/container), water (20L/container), or chloropicrin (6 ml/container). Sealing was done with a polyethylene film for three weeks. After that, *Fusarium oxysporum* f. sp. *lycopersici* in an inoculum pack preliminarily buried in the soil was detected at ca. 6.5 and 2.9 (Log CFU/g dry matter) in non-treated control and water treatment but was not detected in chloropicrin, ethanol, and wheat bran treatment. Each treatment soil was separated into two small plastic containers and infested with the pathogen ( $3 \times 10^5$  cells/g dry soil). Two-week-old tomato seedlings (cv. Regina) were transplanted to the containers and kept in glass house. Four weeks cultivation was done twice successively with the same soil.

In the 1<sup>st</sup> cultivation, severe wilting was observed only in non-treated infested control and chloropicrin treatment but not in water, wheat bran and ethanol treatment (Fig. 3). In the ethanol treatment, disease development was not observed in the 2<sup>nd</sup> cultivation (Fig. 3). Soil suppressiveness was drastically weakened by chloropicrin fumigation but was maintained (induced) by ethanol treatment in both experiments. The results of wheat bran treatments were inconsistent between the experiments. Contribution of soil microorganisms to the soil suppressiveness was suggested but more precise analysis was needed.

In this study, it was suggested that disease suppression by ABSDs might be due to both reducing pathogen density and induction/maintenance of soil suppressiveness, especially with *Fusarium* wilt. Consequently, it is expected that application of ABSDs to Buffer Zone may reduce risk for re-infestation by pathogens to fumigated zone.

This study was supported by grants “Research and development projects for application in promoting new policy of Agriculture Forestry and Fisheries (2019)” from the Japanese Ministry of Agriculture, Forestry and Fisheries and “The Environmental Research and Technology Development Fund” from the Japanese Ministry of the Environment.

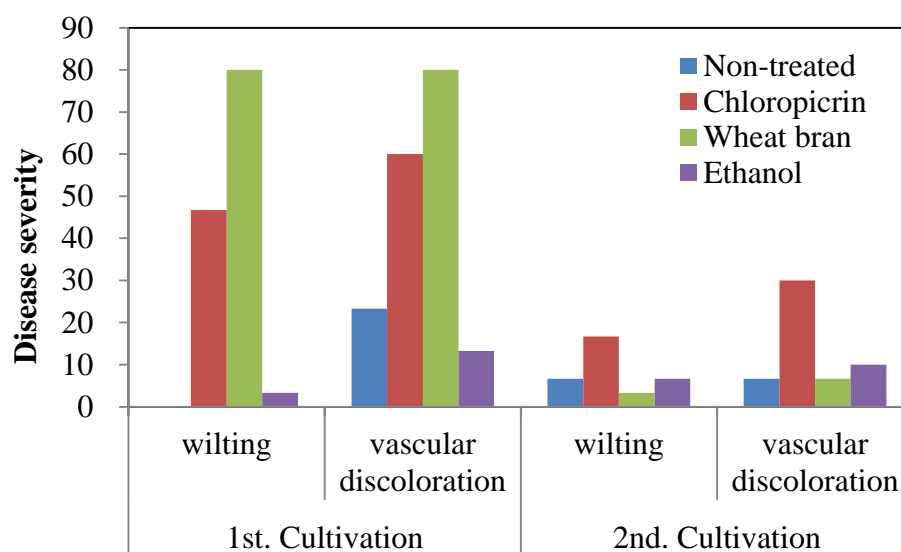


Fig. 1 Disease development by *Fusarium oxysporum* f. sp. *lycopersici* that was artificially inoculated after soil disinfestation (in 2010).

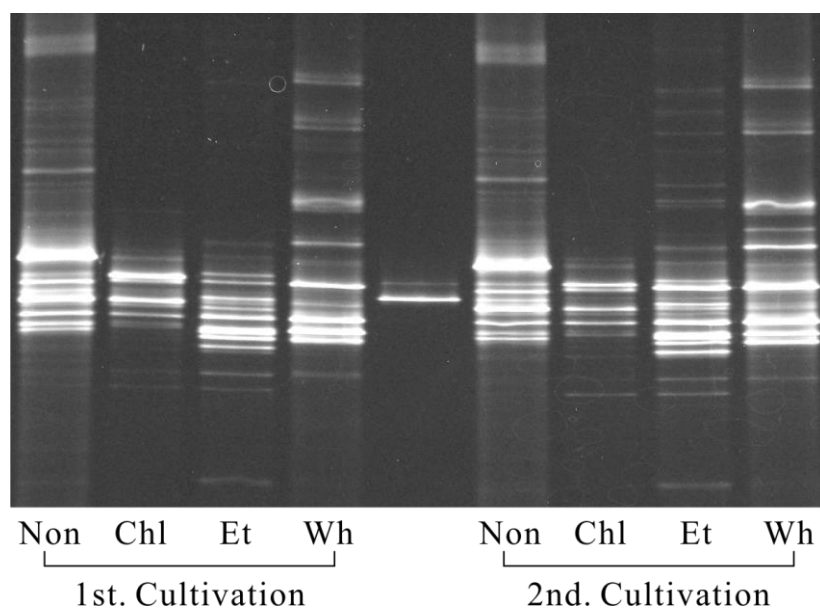


Fig. 2 PCR-DGGE analysis on fungal community structure.  
Non: Non-treated control, Chl: Chloropicrin treatment,  
Et: Ethanol treatment, Wh: Wheat bran treatment

Table 1 The number of soil fungi.

Treatments	Pre-cultivation	After 1 <sup>st</sup> cultivation	After 2 <sup>nd</sup> Cultivation
Non-treated	4.0 (0.0)*	4.4 (0.2)	5.0 (0.0)
Chloropicrin	2.9 (0.1)	3.1 (0.1)	3.7 (0.1)
Wheat bran	4.7 (0.1)	4.2 (0.0)	4.6 (0.0)
Ethanol	4.7 (0.1)	4.4 (0.1)	4.6 (0.1)

\* Log CUF/g dry soil ( $\pm$ SE)

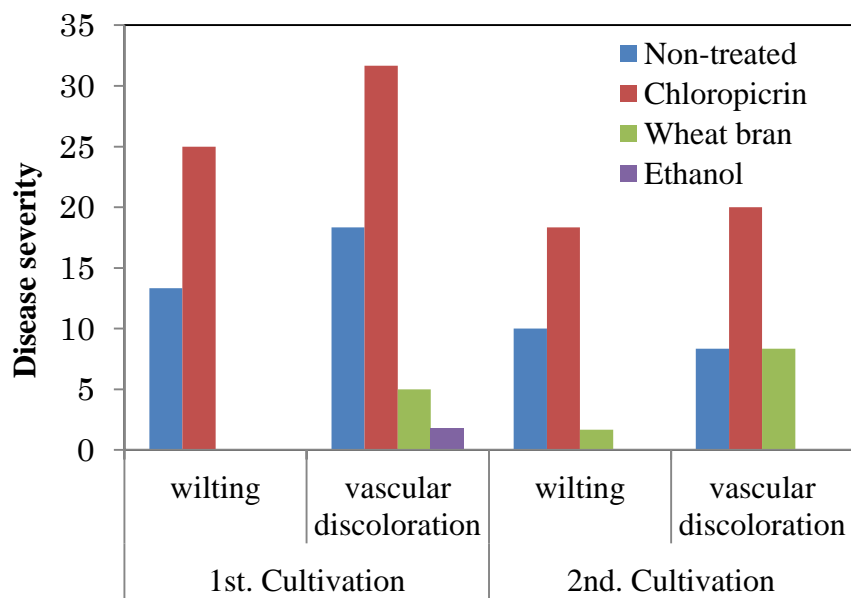


Fig. 3 Disease development by *Fusarium oxysporum* f. sp. *lycopersici* that was artificially inoculated after soil disinfestation (in 2013).

## References

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