# Effects of Soil Biofumigation on Soil Amendment, Suppression of Ralstonia solanacearum, and Tomato Growth: Postprint

**Authors:** Wu Chaorong, Huang Fei, Gao Yang, Mao Yihang, Cai Kunzheng

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## Abstract

Aiming at the current problems of soil continuous cropping obstacles and degradation caused by intensive and monoculture cropping, soil incubation and field experiments were conducted to investigate the effects of soil bio-disinfection (adding 2% rice bran, wheat bran, and tea seed bran to soil, then covering with plastic film, with no material addition and no covering as control) on soil properties, bacterial wilt control, tomato growth, yield, and quality, in order to provide references for theoretical research and practical application of soil bio-disinfection. The results showed that, compared with the control, different soil bio-disinfection treatments significantly increased soil temperature, pH, and electrical conductivity, decreased soil Eh, and reduced the population of bacterial wilt pathogen in soil by 97.27%~99.14%; simultaneously significantly increased soil organic matter, total nitrogen, alkali-hydrolyzable nitrogen, and available potassium contents, while having no significant effect on total phosphorus and total potassium. Different soil bio-disinfection treatments significantly reduced bacterial wilt incidence by 29.41%~42.65%. Furthermore, soil bio-disinfection significantly increased Fv/Fm in tomato leaves, but had no significant effect on photosynthetic parameters including net photosynthetic rate, transpiration rate, stomatal conductance, and intercellular CO2 concentration; significantly increased tomato plant height (16.90%~29.15%) and yield (41.41%~56.25%); fruit sugar-acid ratio and soluble sugar content also increased. The bio-disinfection treatment with wheat bran addition showed the best effect in increasing pH, controlling bacterial wilt, and improving yield. In summary, as a non-chemical soil disinfection method, soil bio-disinfection demonstrated considerable advantages in soil improvement, control of soil-borne disease bacterial wilt, and promotion of tomato growth, and is worthy of popularization and application.

# Full Text

## Effect of Biological Soil Disinfestation on Soil Improvement, Ralstonia solanacearum Suppression, and Tomato Growth

**WU Chaorong, HUANG Fei, GAO Yang, MAO Yihang, CAI Kunzheng**

Key Laboratory of South China Agro-environment in Tropic, Ministry of Agriculture / College of Natural Resources and Environment, South China Agricultural University, Guangzhou 510642, China

### Abstract

Intensive and monoculture cropping systems have caused severe soil degradation and continuous cropping obstacles in agricultural production. This study investigated the effects of biological soil disinfestation (BSD) on soil properties, bacterial wilt control, and tomato growth and yield through both incubation and field experiments. The BSD treatment involved incorporating 2% rice bran, wheat bran, or tea seed bran into soil followed by plastic film mulching, with a non-amended, non-mulched control for comparison. Results demonstrated that all BSD treatments significantly increased soil temperature, pH, and electrical conductivity (EC) while decreasing soil Eh (redox potential) and reducing Ralstonia solanacearum populations by 97.27%-99.14% compared to the control. The treatments also significantly enhanced soil organic matter, total nitrogen, alkaline hydrolysis nitrogen, and available potassium contents, though they had no significant effect on total phosphorus and total potassium. Bacterial wilt incidence was reduced by 29.41%-42.65% across the BSD treatments. Furthermore, BSD significantly increased the chlorophyll fluorescence parameter Fv/Fm in tomato leaves but did not significantly affect photosynthetic parameters including net photosynthetic rate, transpiration rate, stomatal conductance, or intercellular $CO_2$ concentration. Plant height increased by 16.90%-29.15% and yield by 41.41%-56.25% under BSD, with improvements also observed in fruit sugar-acid ratio and soluble sugar content. Among the three organic amendments, wheat bran addition showed the best performance in increasing pH, controlling bacterial wilt, and enhancing yield. Overall, as a non-chemical soil disinfestation method, BSD demonstrates considerable potential for soil improvement, control of soil-borne bacterial wilt disease, and promotion of tomato growth, warranting broader application in agricultural practice.

**Keywords:** Biological soil disinfestation; Bacterial wilt; Soil nutrients; Photosynthetic characteristics; Tomato growth; Tomato yield

## Introduction

Tomato (*Solanum lycopersicum*), renowned as the "king of vegetables" for its nutritional value, ranks among the world' s most productive vegetable crops and represents a major cultivated vegetable species in China. Since 1978, tomato cultivation area in China has expanded continuously, with total production increasing rapidly. By 2011, China' s tomato planting area reached 980,000 hectares with a total output of 48 million tons, ranking first globally. However, the intensification, scaling, and monoculture of tomato production have caused serious soil degradation and continuous cropping obstacles that severely constrain productivity. Bacterial wilt, a soil-borne disease caused by *Ralstonia solanacearum*, has become particularly severe and difficult to control, earning the moniker "plant cancer." This disease predominantly occurs in regions south of the Yangtze River, especially in South China, causing substantial economic losses to agricultural production.

In recent years, biological soil disinfestation (BSD) has gained widespread application internationally as a method for controlling soil-borne diseases and improving soil quality. This approach not only effectively manages soil-borne pathogens and weeds but also utilizes agricultural waste materials such as crop straw and animal manure to enhance soil quality. Numerous studies have investigated BSD for controlling soil-borne diseases and improving soil properties. Research has shown that BSD can effectively reduce nitrate accumulation in continuous cropping systems, increase soil pH, enhance certain soil nutrients, and control weeds, soil pests, and fungal or bacterial pathogens. Consequently, BSD has proven superior to other disinfestation methods in ameliorating soil salinization and acidification, controlling soil-borne pests and diseases, and improving soil fertility, thereby ensuring stable crop yields. As an efficient, broad-spectrum, and environmentally friendly alternative to chemical fumigation, BSD is gradually being promoted worldwide. However, most current research has focused on disease resistance against a limited number of soil-borne pathogens, with fewer reports on impacts regarding soil nutrients, crop growth, yield, and product quality. Given the severe problems of deteriorated soil physicochemical properties, high incidence of bacterial wilt, and reduced crop yield and quality resulting from long-term continuous vegetable cropping, this study combined incubation and field experiments to investigate the effects of BSD on *Ralstonia solanacearum* suppression, soil properties, and tomato growth and quality, providing a theoretical and practical basis for BSD application.

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### 1.1 Soil Materials and Experimental Site

Both the field and incubation experiments used soil collected from the same site in Zhucun Town, Zengcheng District, Guangzhou, Guangdong Province (113.70°E, 23.28°N). The soil was acidic and clayey, with the following physicochemical properties: organic matter 16.4 $g \cdot kg^{-1}$, total nitrogen 0.81 $g \cdot kg^{-1}$, total phosphorus 1.12 $\mathrm{g \cdot kg^{-1}}$, total potassium 27.9 $\mathrm{g \cdot kg^{-1}}$, alkaline hydrolysis nitrogen 82.65 $\mathrm{mg \cdot kg^{-1}}$, available phosphorus 72.80 $\mathrm{mg \cdot kg^{-1}}$, available potassium 120.28 $\mathrm{mg \cdot kg^{-1}}$, pH 4.55, water content 15.78%, and electrical conductivity (EC) 0.062 $\mathrm{mS \cdot cm^{-1}}$. The experimental field had a history of rice (*Oryza sativa*)-tomato rotation for over five years, with bacterial wilt occurring annually in tomatoes. The disease incidence varied yearly due to different factors but showed an overall increasing trend, with the most recent incidence exceeding 45.0%.

Three organic materials were used for BSD in both experiments: rice bran, wheat bran, and tea seed bran, all in coarse powder form and purchased from Zhucun Town, Zengcheng District, Guangzhou. The carbon and nitrogen contents and C/N ratios of these materials are presented in . The tomato cultivar used in the field experiment was 'Jianong T018' produced by Xi' an Hongfeng Seed Co., Ltd. The *Ralstonia solanacearum* strain used was race 1, biovar III, provided by the College of Horticulture, South China Agricultural University.

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## 1.2 Experimental Design

**1.2.1 Field Experiment** The field experiment was conducted from September 25, 2015, to April 8, 2016. Four treatments were established: control (CK) and three BSD treatments with rice bran (ADR), wheat bran (ADW), and tea seed bran (ADT) additions, each with four replications. A randomized block design was employed, with each plot measuring 12.0 m × 1.0 m (12.0 $\mathrm{m^2}$). Organic materials were applied at a rate of 1.0 kg per square meter. The procedure involved: (1) applying rice bran, wheat bran, or tea seed bran to the respective treatment plots, with no material added to the control; (2) thorough mechanical mixing, followed by irrigation to full saturation; (3) covering with plastic film to create anaerobic, moisture-retaining conditions and weed control for three weeks of biological disinfestation; and (4) removing the film and allowing a two-week stabilization period before transplanting. Tomatoes were planted in double rows with 24.0 cm spacing between plants and 30.0 cm between rows, with 100 seedlings (3–4 true leaves) transplanted per plot. Standard unified field management practices were applied after transplanting.

During the treatment period, soil temperature at 20 cm depth was measured every five days at 13:00 (the daily temperature peak). Immediately after the three-week treatment, fresh soil samples were collected to determine oxidation-reduction potential (Eh), while additional samples were air-dried and sieved for pH and EC measurements. After tomato transplanting, chlorophyll content was measured in upper, middle, and lower leaves during the flowering and fruit-setting stage. The third leaf from the top was selected for measuring Fv/Fm (PSII photochemical efficiency) and photosynthetic parameters. Bacterial wilt incidence and tomato yield were recorded throughout the growth period, and fruit quality was assessed using fruits from the second and third clusters at maturity.

**1.2.2 Soil Incubation Experiment** The incubation experiment included four treatments: control (CK) and BSD with rice bran (ADR), wheat bran (ADW), and tea seed bran (ADT), each replicated four times. For each treatment, 2.0 kg of test soil was placed in a pot, inoculated with 20.0 mL of *Ralstonia solanacearum* suspension, and amended with 40.0 g (2%) of the respective organic material. After thorough mixing and addition of 560.0 mL water to achieve moist conditions, the soil was sealed in 20 cm × 30 cm self-sealing bags. The control received no organic material, water, or sealing. All treatments were placed outdoors for three weeks from May 11 to June 2, 2016.

Soil temperature was monitored during the treatment period. After three weeks, bags were opened and fresh soil samples were immediately collected for Eh and *Ralstonia solanacearum* quantification. Additional samples were air-dried, sieved, and used for determining pH, EC, and nutrient contents.

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## 1.3 Measurement Methods

**1.3.1 Soil Temperature, pH, EC, and Eh** Soil temperature was measured according to national standard GB7839—87 using a portable insertion thermometer. For pH determination, air-dried soil was mixed with $1.0\ \mathrm{mol \cdot L^{-1}}$ KCl solution at a 1:5 ratio, shaken for 1 hour, left to stand for 1 hour, and measured using a Leici PHS-3C pH meter. EC was measured in a 1:5 soil-to-deionized water ($\mathrm{EC} < 0.2\ \ \mathrm{S \cdot cm^{-1}}$) extract after 20 minutes of equilibration and filtration using a SIN CT-TDS3031 conductivity pen. Soil Eh was determined using an SX712 ORP meter at a soil-to-water ratio of 5:1.

**1.3.2 Soil Nutrients and Organic Material Carbon/Nitrogen Content** Soil organic matter, total nitrogen, phosphorus, potassium, alkaline hydrolysis nitrogen, available phosphorus, available potassium, and organic material total carbon and nitrogen were measured according to reference [8]. Organic matter was determined by high-temperature external heating potassium dichromate oxidation-volumetric method; total nitrogen by Kjeldahl distillation-titration method; total phosphorus by sodium hydroxide fusion-molybdenum antimony anti-colorimetric method; total potassium by sodium hydroxide fusion-flame atomic absorption spectrophotometry; alkaline hydrolysis nitrogen by alkaline hydrolysis diffusion method; available phosphorus by hydrochloric acid-ammonium fluoride extraction-molybdenum antimony anti-colorimetric method; and available potassium by ammonium acetate extraction-flame atomic absorption spectrophotometry.

**1.3.3 Soil Ralstonia solanacearum Quantification** Soil *Ralstonia solanacearum* populations were determined using 2,3,5-triphenyltetrazolium chloride (TTC) medium via plate counting. The calculation formula was: number of bacteria per gram dry soil = (average colony count of three replicates at each dilution × dilution factor) / (soil sample mass × soil water content).

**1.3.4 Disease Investigation** In the field experiment, bacterial wilt incidence was observed and recorded every three days starting two weeks after tomato transplanting until plants died completely, with a total recording period of 60 days.

**1.3.5 Tomato Chlorophyll Content, Chlorophyll Fluorescence, and Photosynthetic Parameters** Chlorophyll content was measured using a SPAD-502-PLUS portable chlorophyll meter (Konica Minolta). The Fv/Fm (PSII photochemical efficiency) value and photosynthetic parameters were determined using an OS-30P chlorophyll fluorometer (INC, UK) and Li-6400 photosynthesis system, respectively. Photosynthetic parameters included net photosynthetic rate, transpiration rate, stomatal conductance, and intercellular $CO_2$ concentration. All parameters were measured in five replicate plants per treatment.

**1.3.6 Tomato Yield and Quality** Yield was calculated based on the total harvestable produce from each plot until completion of harvest. Soluble sugar content was determined by anthrone colorimetry, protein content by Coomassie brilliant blue G-250 method, nitrate content by colorimetry, organic acid content by sodium hydroxide titration, and vitamin C content by xylene extraction colorimetry, all according to reference [9].

**1.4 Data Analysis** Experimental data were processed and analyzed for significant differences among treatments using one-way ANOVA with LSD test at $P < 0.05$ in SPSS 18.0. Figures were created using Microsoft Excel 2010.

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## Results

### 2.1 Effects of BSD on Soil Temperature, pH, EC, and Eh

Both incubation ([Figure 1: see original paper]A) and field experiments ([Figure 1: see original paper]B) showed that BSD treatments significantly increased soil temperature at 20 cm depth by 7-8°C and 5-6°C, respectively, compared to the control, with no significant differences among the three BSD treatments.

The effects of BSD on soil pH, EC, and Eh are shown in [Figure 2: see original paper]. All BSD treatments significantly increased soil pH by 14.89%-19.11% compared to CK in both experiments ([Figure 2: see original paper]A). In the incubation experiment ([Figure 2: see original paper]B), BSD treatments significantly increased soil EC by 4-6 fold, whereas in the field experiment, only the

ADW treatment showed significantly higher EC than the control. BSD treatments markedly reduced soil Eh to negative values in the incubation experiment, while in the field experiment, only ADR showed significantly lower Eh than CK ([Figure 2: see original paper]C).

## 2.2 Effects of BSD on Soil Nutrients and Ralstonia solanacearum Populations

After three weeks of incubation, changes in soil nutrient contents are presented in . Compared to the control, all BSD treatments significantly improved soil nutrients, increasing organic matter by 32.11%-82.95%, total nitrogen by 12.08%-59.36%, and available potassium by 1.28-2.43 fold, while showing no significant effects on total phosphorus, total potassium, or available phosphorus. Additionally, soil *Ralstonia solanacearum* populations were significantly reduced by 97.27%-99.14% across all BSD treatments compared to CK ([Figure 3: see original paper]).

## 2.3 Effects of BSD on Leaf Photosynthesis, Chlorophyll Content, and Chlorophyll Fluorescence Parameter Fv/Fm

Compared to the control, BSD treatments had no significant effects on tomato leaf net photosynthetic rate, stomatal conductance, intercellular $CO_2$ concentration, or transpiration rate. However, Fv/Fm values were higher in all BSD treatments than in CK, with ADR and ADW showing significant differences (). Furthermore, ADW significantly increased SPAD chlorophyll values, while ADR and ADT showed no significant differences from CK in chlorophyll content.

## 2.4 Effects of BSD on Tomato Growth, Bacterial Wilt Resistance, and Yield

During the late growth stage (fruit expansion period), all BSD treatments significantly increased plant height, with ADW showing the greatest improvement at 29.2% over CK, though no significant effects on stem diameter were observed ([Figure 4: see original paper]A, 4B). During the disease monitoring period, all BSD treatments effectively reduced bacterial wilt incidence, with ADR, ADW, and ADT decreasing incidence by 42.65%, 40.41%, and 29.41%, respectively, at day 56 compared to CK ([Figure 4: see original paper]C). Correspondingly, tomato yield increased by 45.31%, 56.25%, and 41.41%, respectively, with ADW showing the highest increase ([Figure 4: see original paper]D).

## 2.5 Effects of BSD on Tomato Quality

Tomato quality measurements across treatments are shown in . Compared to CK, ADT significantly increased soluble sugar content and sugar-acid ratio, while other BSD treatments showed non-significant improvements in vitamin C and soluble protein content. No significant effects were observed on organic acid or nitrate contents.

## Discussion

The development of protected vegetable cultivation has transformed tomato production from seasonal to year-round systems. However, high-density multiple cropping, excessive chemical fertilizer and pesticide use, and improper field management have exacerbated continuous cropping obstacles and soil-borne bacterial wilt. Bacterial wilt occurrence is closely associated with various soil physicochemical factors, including temperature, moisture, and pH. The disease thrives in high-temperature, high-humidity, and acidic environments, with an optimal temperature range of 30–37°C and a disease occurrence range of 10–40°C. Our results demonstrate that BSD treatments effectively increased soil temperature and pH while decreasing Eh. In the incubation experiment, BSD raised soil temperature to near 45°C and reduced Eh to negative values, creating a high-temperature, strongly reductive, or anaerobic environment that inhibited *Ralstonia solanacearum* proliferation, leading to substantial reductions in pathogen populations and disease incidence. The temperature increase resulted primarily from solar heating and chemical decomposition of organic materials, while the pH effects were closely related to the type of organic amendment applied. Using rice bran, wheat bran, and tea seed bran as carbon sources, BSD increased soil pH by 14.89%-19.11%, possibly due to the initially low pH of the test soil, consistent with findings by Hewavitharana et al. [11].

BSD treatments significantly reduced soil Eh in the incubation experiment, aligning with previous reports [12–14]. This reduction likely occurred because the sealed, anaerobic conditions promoted vigorous activity of anaerobic microorganisms, producing abundant reductive substances that created strongly reductive soil conditions. However, Eh changes were less pronounced in the field experiment, possibly due to incomplete sealing, shorter anaerobic duration, and lower organic material application rates [7,15]. The significant increase in soil EC under BSD primarily resulted from increased release of salts and metal ions. During BSD, mineralization of organic materials and strongly reductive conditions increased concentrations of ferrous iron ($Fe^{2+}$), manganese ($Me^{2+}$), and ammonium ($NH_4{}^+$) [16,17], which may enhance suppression of soil-borne pathogens.

During BSD, added organic materials undergo mineralization and degradation, significantly increasing soil nutrient contents. Studies have shown that nutrient release rates from straw incorporation follow the order: potassium > phosphorus > carbon > nitrogen [18,19]. Our three-week BSD treatments significantly increased soil organic matter, total nitrogen, alkaline hydrolysis nitrogen, and available potassium compared to the control, consistent with results from Gu et al. [13]. The lack of significant effects on total potassium, total phosphorus, and available phosphorus may be attributed to low potassium and phosphorus contents in the added organic materials. Previous research indicates that BSD effectively increases total carbon and nitrogen while decreasing nitrate nitrogen [20,21], and that wheat straw flooding promotes release of dissolved organic carbon (DOC) and dissolved organic nitrogen (DON) in paddy soil solution [22].

Chlorophyll fluorescence reflects the impact of stress factors on photosynthesis, with Fv/Fm commonly used as an indicator of environmental stress severity. Under stress conditions, plants typically exhibit decreased Fv/Fm, photosynthetic rate, and transpiration rate [23,24]. In our study, slightly higher or equal chlorophyll content and Fv/Fm values in BSD treatments may be related to improved soil nitrogen availability [25]. Additionally, the lack of significant differences in the four photosynthetic parameters (net photosynthetic rate, stomatal conductance, intercellular $CO_2$ concentration, and transpiration rate) indicates that BSD does not significantly affect tomato photosynthesis.

BSD promotes stable and increased crop production primarily through controlling soil-borne pests and diseases, followed by improving other soil environmental factors. Shrestha et al. [4] conducted a meta-analysis of 123 studies on BSD effects on crop yield, finding that all BSD treatments significantly increased yield compared to controls. Butler et al. [26] used molasses as a carbon source and found BSD ensured pepper (*Capsicum annuum*) and eggplant (*Solanum melongena*) yields equal to or greater than those achieved with methyl bromide (MeBr) chemical fumigation. Li et al. [27] compared solar disinfestation (physical), lime nitrogen-wheat straw (biological-chemical), and dazomet (chemical) soil disinfestation methods, finding all three promoted cucumber (*Cucumis sativus*) growth and increased yield. Our study demonstrates that BSD improves soil quality, enhances certain soil nutrients, does not significantly affect tomato photosynthesis, effectively mitigates bacterial wilt damage, reduces disease incidence, and significantly promotes tomato growth and yield. Additionally, we observed that BSD-treated tomato plants showed delayed wilting compared to CK when exposed to cold damage during fruit expansion, suggesting enhanced cold tolerance.

In conclusion, BSD plays a beneficial role in ameliorating continuous cropping obstacle soils and promoting crop growth. Compared to the control, different BSD treatments significantly increased soil pH and temperature, decreased soil Eh and *Ralstonia solanacearum* populations, enhanced soil nutrient contents, promoted tomato plant growth, improved resistance to bacterial wilt, and substantially increased tomato yield. Moreover, BSD treatments showed some positive effects on leaf chlorophyll content and fruit quality. Among the three organic amendments, wheat bran demonstrated the best performance in controlling bacterial wilt and increasing yield, making it worthy of promotion in agricultural production.

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