ISSN 1001-0742
CN 11-2629/X

JOURNAL OF ENVIRONMENTAL SCIENCES

May 1, 2015 Volume 31
www.jesc.ac.cn

Real-world diesel vehicle emission factors for China

ELSEVIER
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Journal of Environmental Sciences Volume 31 2015

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Effects of water regime, crop residues, and application rates on control of *Fusarium oxysporum* f. sp. *cubense*

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ARTICLE INFO

Article history:
Received 10 August 2014
Revised 9 October 2014
Accepted 26 November 2014
Available online 11 March 2015

Keywords:
Biological soil disinfestation
Banana *Fusarium* wilt
Organic amendments
Water regime treatment duration
Treatment duration

ABSTRACT

Biological soil disinfestation is an effective method to control soil-borne disease by flooding and incorporating with organic amendments, but field conditions and resources sometimes limited its practical application. A laboratory experiment was conducted to develop practice guidelines on controlling *Fusarium* wilt, a widespread banana disease caused by *Fusarium oxysporum* f. sp. *cubense* (FOC). FOC infested soil incorporated with rice or maize straw at rates of 1.5 tons/ha and 3.0 tons/ha was incubated under flooded or water-saturated (100% water holding capacity) conditions at 30°C for 30 days. Results showed that FOC populations in the soils incorporated with either rice or maize straw rapidly reduced more than 90% in the first 15 days and then fluctuated till the end of incubation, while flooding alone without organic amendment reduced FOC populations slightly. The rapid and dramatic decrease of redox potential (down to –350 mV) in straw-amended treatments implied that both anaerobic condition and strongly reductive soil condition would contribute to pathogen inactivation. Water-saturation combined with straw amendments had the comparable effects on reduction of FOC, indicating that flooding was not indispensable for inactivating FOC. There was no significant difference in the reduction of FOC observed in the straw amendments at between 1.5 and 3 tons/ha. Therefore, incorporating soil with straw (rice or maize straw) at a rate of 3.0 tons/ha under 100% water holding capacity or 1.5 tons/ha under flooding, would effectively alleviate banana *Fusarium* wilt caused by FOC after 15-day treating under 30°C.

Introduction

Banana is one of the most important agricultural crops in the world and serves as a major source of income in many developing countries (Dita et al., 2010). *Fusarium* wilt of banana (*Musa* spp.) is considered to be the most serious fungal disease attacking banana varieties (O'Donnell et al., 1998; Wang et al., 2013). The disease is known to be commonly caused by *F. oxysporum* f. sp. *cubense* (FOC) (Snyder and Hansen, 1940). Symptoms of *Fusarium* wilt begin with yellowing and wilting of the older leaves, and then spread to the younger leaves till the death of whole plant. FOC is a typical soil-borne pathogen that produces chlamydospores as a resting propagule. They are thick-walled and resistant to environmental fluctuations, enabling the pathogen to survive in soil a long time

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http://dx.doi.org/10.1016/j.jes.2014.11.007
1001-0742/

without host plants (Momma et al., 2010). Once being infested by FOC in soil, the susceptible banana varieties cannot be successfully replanted for nearly decades (Stover and Ploetz, 1990). Hence, the banana production has been dramatically reduced in many countries, ranging from the Central America to Asian (Ploetz, 2006).

Aiming to control FOC, many attempts have been conducted, such as planting resistant banana varieties, applying chemical pesticides and bio-control agents, and rotating crops (Stover and Ploetz, 1990; Alabouvette et al., 2009; Helslop-Harrison and Schwarzacher, 2007). A new approach, called biological soil disinfestations (BSDs) or anaerobic soil disinfestations (ASDs) or reductive soil disinfestations (RSDs) is proposed to control a broad spectrum of soil-borne pathogens (including FOC) and nematodes (Blok et al., 2000; Momma et al., 2006; Shennan et al., 2007; Mowlick et al., 2013). This approach is characterized by incorporating easily decomposable organic amendments, flooding soil through irrigation, and covering with plastic film for a period to create anaerobic conditions.

While the BSD is promising in controlling banana wilt disease, several aspects of problems hinder its practical application. First, flooding requires large amount of water and limited to fields, which are able to maintain standing water, layer and are equipped with convenient hydraulic and irrigation facilities (Blok et al., 2000; Gamliel et al., 2000; Katan, 2000). Soil texture and profile structure can influence the flooding effect. Organic soils with fine texture have a tendency to retain water, while coarse texture soils tend to drain water (Snyder, 1987). Flooding might not be practical in hilly areas where standing water layer could not be easily formed and in fields whose soils are permeable intensively with limitation of water resource. Moreover, considering water is a valuable resource, a more efficient and conservative way should be developed. Previous researches have revealed that saturating the soil to its maximum water holding ability is an effective alternative approach, because it can not only fill soil voids with water and restrict gas exchange as flooding does, but also conserve water and reduce nutrient runoff (Momma et al., 2010; Muramoto et al., 2008; Snyder, 1987). However, other studies found that merely saturating the soil was not effective in inhibiting some soil pathogens such as *Pythium* spp. (Snyder, 1987). Therefore, further work is needed to clarify this point.

Second, BSD requires the incorporation of a great number of organic amendments into soil. The incorporation rate of 10 tons/ha or even more was reported (Butler et al., 2012a; Momma, 2008; Momma et al., 2006, 2013; Subbarao et al., 1999). Although a variety of organic materials, including molasses, wheat bran, broccoli, ethanol, cover crops, animal and green manure could be used for BSD approach (Momma et al., 2011; Núñez-Zofío et al., 2011; Shinmura, 2002), they are not easily accessible all the time and are not economical due to transportation and purchase expense. On the other hand, crop residues have long been regarded as waste agricultural byproducts and even as contamination sources. Open-field burning of crop residues has induced serious atmospheric pollution in China (Zhang and Zhang, 1999). Also, *in situ* incorporation of crop residues is not adoptable, because the duration of interval between two crops is too short in the areas where multiple cropping is practiced, and it may increase the conductive to foliar diseases and reduce rough crop yields (Buresh and Sayre, 2007; Gadde et al., 2009; Hrynchuk, 1998). Therefore, if crop residues could be used as organic amendments in BSD, it would be possible to establish an environment-friendly straw disposal strategy to take the place of open-field burning.

In this study, in order to develop a more practical and economical BSD method to control *Fusarium* wilt, a laboratory experiment was conducted to determine: (1) Whether maize or rice straw has the potential to be used for BSD approach for controlling banana *Fusarium* wilt disease? (2) Whether water-saturation can replace flooding to reduce population of FOC, a pathogen of banana *Fusarium* wilt disease? and (3) How many days of water-saturation is the minimum to reduce the FOC population maximally?

1. Materials and methods

1.1. Soil and crop residues

The soil for the laboratory experiment was collected from the farm of Hainan Wanzhong Co., Ltd., Hainan, China. The field had been planted with banana for more than 10 years and *Fusarium* wilt disease incidence had spread most area of the farm, which was no longer suitable for banana growth. Because of undulating terrain, a standing water layer was hard to be formed for BSD treatment. The soil sample was collected from a field, which was abandoned for two years due to the *Fusarium* wilt disease incidence more than 50%. The soil was sand loamy, developed from red dry soil, with pH value of 6.55, organic matter content of 4.95 g/kg, and total N content of 1.29 g/kg. Before the experiment, the soil was sieved through a 2 mm sieve. Locally collected rice and maize straw were selected as organic materials for BSD treatment. The rice straw contained 42.5% C and 0.77% N (C:N ratio of 55:1, *m*/*m*), and the maize straw contained 47.1% C and 0.68% N (C:N ratio of 69:1, *m*/*m*). Both two kinds of straw were air dried for about one week, chopped into approximately 2-3 cm pieces, grounded into powders and finally sieved through a 2 mm sieve.

1.2. Experiment design

In the laboratory, a set of pots (1.5 L volume) were filled with 500 g prepared soil (soil depth: 5.5 cm, surface area: 78.5 cm^2) and then treated as follows: (1) non-amended and non-flooded (control CK); (2) non-amended but flooded (flood control); (3) flooded and amended with rice straw at a rate of 1.5 tons/ha (Flood + LR) (The conversion between weight and area units was based on the soil bulk density of 1.2 g/cm^3); (4) flooded and amended with rice straw at a rate of 3.0 tons/ha (Flood + HR); (5) flooded and amended with maize straw at a rate of 1.5 tons/ha (Flood + LM); (6) flooded and amended with maize straw at a rate of 3.0 tons/ha (Flood + HM); (7) water saturated and amended with rice straw at a rate of 3.0 tons/ha (Saturate + HR); (8) water saturated and amended with maize straw at a rate of 3.0 tons/ha (Saturate + HM). Each treatment had three replicates. For the incorporation rate of 1.5 tons/ha and 3.0 tons/ha, 0.32 g and 0.64 g straw was respectively added into each pot with 500 g soil. After thoroughly mixed with designed amount of crop residues, tapwater was added to soil/water ratio of 1:1 in flooded pots and to reach the water holding capacity (100% WHC) in water-saturated pots. Then, each pot was packed in a plastic self-seal bag and incubated in an incubator at 30°C for 30 days. All treatment pots were completely randomly arranged. Incubated soil was analyzed for soil properties and FOC population on days 0, 5, 10, 15, 20 and 30. Among them, the samples incubated after 30 days was divided into two parts, one was analyzed soil properties immediately and another was air dried at room

temperature for 10 days and then analyzed for soil properties.

1.3. Analyses of soil properties

The redox potential of the soil was measured by an ORP meter equipped with platinum (Pt) electrodes and a glass calomel reference electrode (FJA-16, Institute of Soil Science, Chinese Academy of Sciences, Nanjing, China). The pH was measured in slurry (1:1 V/V with deionized water) by a pH electrode (Mettler Toledo, FE20-FiveEasy, USA). NH_4^+-N and NO_3^--N were extracted with 2 mol/L KCl solution at soil/water = 1:5 (*m*/*V*) by shaking at 250 r/min for 1 hr and measured by a continuous-flow analyzer (San++, Skalar Analytical B.V., Breda, the Netherlands). The water holding capacity of soil was measured following several steps: (1) the soil was fully flooded (keep some water left standing on the soil surface) for at least 2 hr; (2) the standing water was allowed to seep out the soil for 6 hr; and (3) the moisture content of the soil was measured (drying at 105°C for 12 hr).

1.4. Assay of the number of culturable soil microbes

A standard 10-fold dilution method was used to calculate the culturable microbes. Soil suspensions were inoculated into agar plates containing suitable media and counted the live microbes to determine the number in a given population. Here, beef extract peptone medium was used for the growth of bacteria (beef extract 5 g, peptone 10 g, NaCl 5 g, agar 20 g, distilled water 1000 mL), Gause No. 1 medium (soluble starch 20 g, KNO_3 1 g, NaCl 0.5 g, K_2HPO_4 0.5 g, $MgSO_4$ 0.5 g, $FeSO_4$ 0.01 g, $K_2Cr_2O_7$ 0.1 g, agar 20 g, distilled water 1000 mL, pH 7.2) was used for the growth of actinomycetes, Martin's bengal rose agar (glucose 10 g, peptone 5 g, KH_2PO_4 1 g, $MgSO_4$ 0.5 g, rose bengal 30 mg, streptomycin 30 mg, agar 20 g, distilled water 1000 mL) was used for the growth of fungi and Komada medium (Komada, 1975) was used for the growth of fusarium (FOC). Inoculated plates were incubated at 30°C for 36 hr for bacteria, at 28°C for 72 hr for fungi and fusarium, and at 30°C for 120 hr for actinomycetes. The total number of microbes were determined by counting the number of colony forming units (CFU) and transformed as log10 (CFU/g dry soil).

1.5. Soil DNA extraction and quantification of FOC

Only the soil samples which were incubated 30 days and followed by 10 days air-dry were used for soil DNA extraction and FOC quantification. Soil DNA was carefully extracted according to the manual instructions (MO BIO UltraClean® Soil DNA Isolation Kit, MO BiO Laboratories, Inc., USA). The DNA quality and quantity were checked on 1.2% agarose gel (ethidium bromide stained) and visualized by UV trans-illumination. The number of FOC internal transcribed spacer (ITS) copies was determined with real-time PCR (Lievens et al., 2006). Real-time PCR amplifications were conducted with a total volume of 20 μL of SYBR® Premix Ex Taq TM (TaKaRa, Takara Biotech company, Dalian, China) on a CRX-96 thermocycler (Bio-Rad Laboratories Inc., Hercules, CA, USA). The total 2 μL of the target DNA extract, 10 μL of SYBR Green premix EX Taq (2×), 0.4 μL of primer ITS1-F and AFP308 (10 μmol/L, Table 1) and 7.2 μL sterilized distilled water were involved in each PCR reaction. Thermal cycling conditions were composed by 2 min at 95°C followed by 40 amplification cycles of 10 sec at 95°C, 15 sec at 58°C and 20 sec at 72°C. Fluorescence was detected at the third stage of each cycle. In order to evaluate amplification specificity, a melt curve analysis was performed at the end of each PCR run. A melt curve profile was established by heating the mixture to 95°C, cooling to 65°C, and slowly heating to 95°C at 0.2°C/sec with continuous measurement of fluorescence.

Table 1 – Primers used in the experiment.

Primer	Sequence (5′–3′) [a]	Product size (bp)	Reference
ITS1-F (F)	CTTGGTCATTTAGAGGAAGTAA	400	Lievens et al. (2006)
AFP308 (R)	CGAATTAACGCGAGTCCCAAC	400	Lievens et al. (2005)

F: forward primer; R: reverse primer.
[a] A GC-rich sequence (GC-) attached to the 5′ end of sequence is indicated.

1.6. Data analysis

All data collected were checked for normality and homogeneity and appropriately transformed before performing statistical analysis. The ANOVA module in SPSS 16.0 (SPSS Inc., Chicago, USA) and SNK tests ($p \leq 0.05$) was used to analyze the variances among different treatments.

2. Results

2.1. Soil properties

Soil redox potential (Eh) was kept around 300 mV in untreated soil (CK) and dropped sharply to −200 mV after 5 days of incubation and then fluctuated between −150 and −250 mV in all flooded and water saturated soils amended with either rice straw or maize straw (Fig. 1a). The lowest Eh was observed in the treatment of Flood + HM, in which Eh decreased from 347 to −307 mV, but it was not differed significantly with those in other soils amended with crop straw ($p > 0.05$). Compared with the crop straw amended groups, the soil Eh decreased more slowly in the treatment of Flood alone, in which soil Eh reached the minimum (−167 mV) on day 15 after incubation and fluctuated at higher value.

The soil pH was obviously elevated after flooded or water saturated (Fig. 1b). Soil pH fluctuated between 7.11 and 7.54 in control pots, while raised to around 8 in all flooded and water saturated soils at the end of the experiment. The incorporation of crop residues increased soil pH directly, but the differences in soil pH between the flooded and water saturated soils became smaller during the incubation and the following air dry. There was no statistically difference in soil pH observed between the flooded and water saturated soils after air dry ($p = 0.85$).

The inorganic N content was very low in the soil, although large amount of N fertilizers was applied during banana growth in the farm. This is probably due to its sandy loam soil texture, low soil organic C and N contents, and the field being

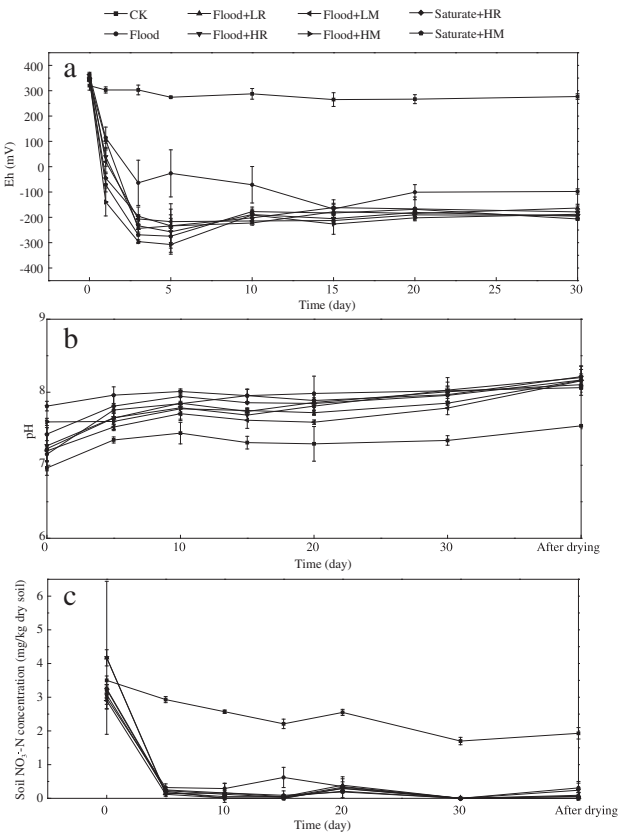

Fig. 1 – Change in soil Eh, pH and nitrate concentration during the 30-day incubation. Bars refer to one standard deviation. The treatments are non-amended and non-flooded (CK), non-amended but flooded (flood), flooded and amended with rice straw at a rate of 1.5 tons/ha (Flood + LR), flooded and amended with rice straw at a rate of 3.0 tons/ha (Flood + HR), flooded and amended with maize straw at a rate of 1.5 tons/ha (Flood + LM), flooded and amended with maize straw at a rate of 3.0 tons/ha (Flood + HR), water saturated and amended with rice straw at a rate of 3.0 tons/ha (Saturate + HR),water saturated and amended with maize straw at a rate of 3.0 tons/ha (Saturate + HM).

abandone. Nitrate content dropped sharply from around 3 mg N/kg to near zero within 5 days of incubation and then kept below 1 mg N/kg in all flooded and water saturated soils (Fig. 1c). Ammonium content was kept below 1 mg N/kg in all the treatments during the incubation (data not shown).

2.2. Actinomyces and bacteria

The population of actinomyces in the untreated control (CK) and flooded alone (Flood) decreased to the minimum first and then recovered almost completely at the end of experiment (Fig. 2a). In contrast, the numbers of actinomyces in the soils amended with crop residues decreased remarkably from the levels of 10^6 in the beginning to 10^5 at the end of the experiment ($p < 0.05$). However, there were no significant differences in the population of actinomyces between the

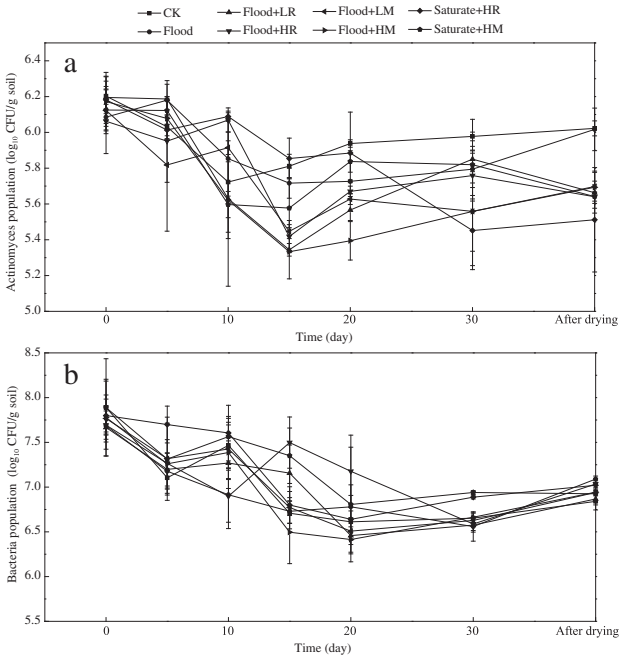

Fig. 2 – Changes in population of actinomyces (a) and bacteria (b) during the incubation and after soil drying. Bars refer to standard deviation.

treatments amended with crop residues at the end of experiment ($p > 0.05$).

Generally, the populations of bacteria in all treatments decreased during the incubation and recovered to certain extent after air dry, but still significantly lower than their initial numeric levels ($p < 0.05$, Fig. 2b). No significant

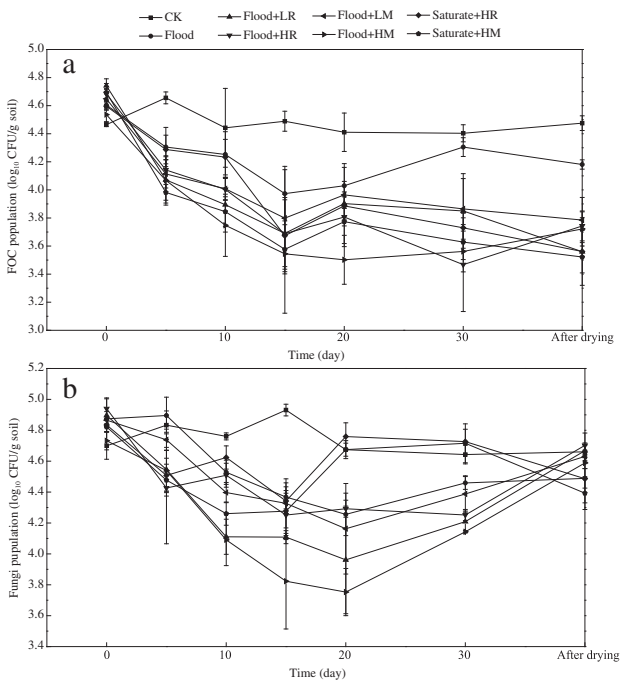

Fig. 3 – Change in population of FOC (a) and fungi (b) during the incubation and after soil drying. Bars refer to standard deviation.

contrasts of bacteria population were noted among the treatments at the end of experiment ($p > 0.05$).

2.3. Pathogen and fungi

The population of FOC in the soil was around 4.0×10^4 CFU/g soil. Flooding alone decreased FOC population at the first 15 days of the incubation, but then recovered to certain extent (Fig. 3a). The numbers of FOC were reduced more sharply in the soils amended with crop residues under either flooded or water saturated conditions than that in flooding alone at the beginning of incubation and dropped to the lowest levels of around 5.0×10^3 CFU/g soil on day 15. After that, the FOC populations in the soils amended with crop residues were slightly fluctuated, but were not statistically distinct with those at day 15 ($p > 0.05$) (Table 2). At the end of experiment, their final viability of FOC in the soils amended with crop residues were all reduced by more than 90% ($p < 0.001$), especially in the treatment of Saturate + HM, which inactivated FOC was up to 96% after the treatment. The final FOC mortality varied from 1.0×10^3 CFU/g soil to 6.3×10^3 CFU/g soil among the treatments amended with crop residues, but they were not statistically differed with each other ($p > 0.05$).

The fungi number in control pots was undoubtedly kept on the same numeric level (*ca.* 6.5×10^4 CFU/g soil) in the experiment. At the beginning of incubation, the changes of fungi populations in the soils mirrored the FOC trends. They were greatly decreased in the beginning of incubation, reached the minimum, and then recovered to certain extent. Though the population at the minimum and days needed to reach the minimum varied among the treatments, their final values were not statistically different ($p > 0.05$, Fig. 3b).

Real-time PCR was applied to the incubated soils after air dry and the results showed that the population of FOC in untreated soil was 6.97×10^5 FOC copies/g soil, followed by the FOC population in soil flooded alone (1.20×10^5 FOC copies/g soil) (Fig. 4). The numbers of FOC in all the soils amended with crop residues range from 4.50 to 8.30×10^4 FOC copies/g soil, being significantly lower than those in the untreated soil and the soil flooded alone ($p < 0.05$). Across all treatments amended crop residues, however, their FOC population was not statistically different ($p > 0.05$).

3. Discussion

Great reductions (the maximum up to 96%) in viable survival FOC populations were found in all amended soils under either flooded or saturated conditions which dropped from the numeric level of 10^4 CFU/g soil to 10^3 CFU/g soil. Application of flooding alone also resulted in a significant reduction of viable pathogens but was not as effective as amended pots. These results clearly illustrate the potential of using crop residues (rice or maize straw) to control FOC, a more economical and practical approach to perform soil disinfestations, and an environmentally friendly method for straw disposal and utilization.

Our results indicated that flooding alone didn't show the same effective suppress on FOC (Fig. 3a). This is in accordance with the findings obtained by Blok et al. (2000) and Mitchell and Alexander (1962). They pointed that direct inactivation of pathogen did not generally occur in soils treated by flooding alone. Blok et al. (2000) reported that flooding alone could rapidly developed anaerobic condition, but the level of oxygen would gradually increase again, probably due to the diffusion compensating over the consumption of oxygen with the depletion of available nutrients in soil (Blok et al., 2000; Momma et al., 2013). Adding organic material not only helps to fortify such an anaerobic condition, but also induces a strong reductive condition as indicated by soil Eh dropping in the experiment (Fig. 1). Both oxygen depletion and strong reductive conditions induced by organic amendments were involved in the mechanism of pathogen inactivation process (Blok et al., 2000). Therefore, amendment of easily decomposable organic materials is imperative in the BSD approach.

In the experiment, two rates of straw (1.5 and 3.0 tons/ha) were incorporated into FOC infested soil. Both of them could greatly inactivate more than 90% of the soil pathogen (FOC) and no obvious differences were noted between them. Indeed, the addition rates of maize and rice straw were relatively low in the present study when compared with previous researches. Usually, 10 tons/ha or more organic materials were amended into soil to control soil-borne disease (Butler et al., 2012a; Momma, 2008; Momma et al.,

Table 2 – Effect of treatments on population density of *Fusarium oxysporum* f. sp. *cubense* (FOC) (CFU/g soil) during the incubation and after soil drying.

Treatment	Day 0	Day 5	Day 10	Day 15	Day 20	Day 30	After drying
Flood + LR	55256 a	11788 b*	7840	4903 cd	7989 bc	7082 bc	3631 d
Flood + HR	48244 a	13872 b*	10080	4876 cde#	6411 c	2934 e	5536 cd
Flood + LM	44563 a	12986 b	10186	6293 c	9212 cb	7323 cb	6100 cb
Flood + HM	34348 a	11686 b	5597	3504 cd	3178 c	3643 cd	5273 d
Saturate + HR	39491 a	19397 b	17107	4716 cd#	7694 c	5369 cd	3640 d
Saturate + HM	49454 a	9597 b*	6998	3776 ce#	5937 c	4250 c	3327 de

Different letters within the same row and sampling data show significant difference (SNK tests ($p \leq 0.05$)).
To make the results more concise, the statistical results of 10-day group were not marked since they were not significantly different with the 5-day group.
* *The sampling data on day 5 at each treatment was strongly significant with its data on day 0 ($p \leq 0.01$).
# The sampling data on day 15 at each treatment was strongly significant with its data on day 10 ($p \leq 0.01$).

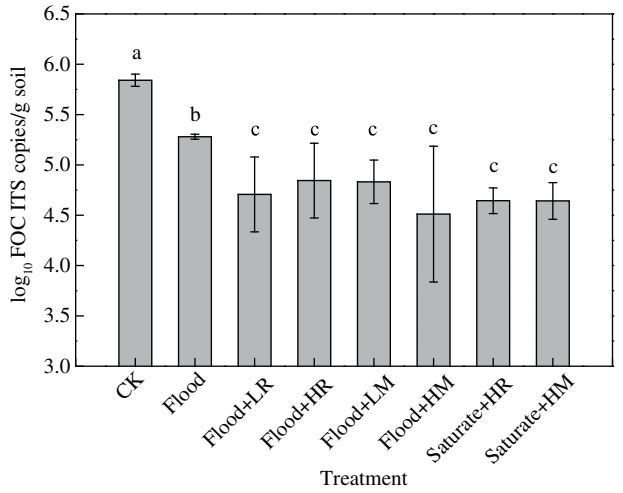

Fig. 4 – Real-time PCR quantification of FOC ITS (Internal Transcribed Spacer) copies measured in each treatment after soil drying. For each group, values followed by the same letter are not significantly different according to SNK tests ($p \leq 0.05$).

2006, 2013; Subbarao et al., 1999). Momma et al. (2010) found that adding more organic material could help to shorten the incubation period and result in higher pathogen mortality. The high effectiveness of rice and maize straw in FOC inactivation in this study was probably due to their higher content of organic matter (rice straw: 78.6%, maize straw: 83.5%) and that the straw residues were dried and grinded into powder before incorporation, which probably made them more easily being decomposed in soil. Furthermore, the coarse texture of studied soil (sandy loamy soil) would make the soil properties, such as Eh and pH, easily changed by BSD treatment.

Our results revealed that in the soils amended with crop residues the incubation under both flooded and water saturated (100% WHC) conditions resulted in large reduction of FOC and no significant difference was observed between the two conditions. The results were consistent with the previous findings that water saturation was sufficient for pathogen control when paired with organic amendments (Blok et al., 2000; Butler et al., 2012b; Momma et al., 2013). The incubation indicated that the changes in soil Eh and pH were also highly comparable under water saturation paired with crop straws and under flooded paired with crop straws (Fig. 1). Hence, instead of flooding which usually consumes a vast amount of water and is limited by resources and locations, water saturation would be an alternative to drive pathogen mortality in BSD approach.

During the 30-day incubation, the fierce changes in soil Eh, pH and population of FOC occurred in the first 15 days in the soils amended with crop straws (Fig. 1). Momma et al. (2006) also suggested that 15-day length of biological soil disinfestations by amendment with wheat bran could greatly suppress the survival of *F. oxysporum* f. sp. *lycopersici* or *Ralstonia solanacearum*. Klein et al. (2011, 2012) incubated soils incorporated with various plant residues for 14 days in laboratory and found that short incubation could effectively control the soil-borne pathogens. The pathogens cannot be detected in the infested soils 9 days after amended with wheat bran, flooded, and covered with plastic film (Momma, 2008; Momma et al., 2013). They detected a small amount of acetic acid at early stage of wheat bran treatment, but the acid volatilized and disappeared very quickly. Compared with the methods such as solarization or flooding alone which usually needs several months or a season period to suppress pathogen, the incubation time in our study was effectively shortened. Admittedly, there were some other researches illustrated that 3–4 week treatment was favorable for pathogen inactivation (Blok et al., 2000; Messiha et al., 2007; Shennan et al., 2007). This discrepancy about treating time is probably depending on the type and amount of organic material being added and the soil properties. Since our experiment was conducted in laboratory, further evaluations in field conditions are necessary.

The mechanism of pathogen disinfestation was not studied specifically in our experiment. However, some indications about the factors involved in the process for inactivation of FOC could be inferred. Direct thermal disinfestation caused by increased soil temperature did not lead to pathogen inactivation, because the population of FOC in the control (CK) did not decrease at all. The temperature in our experiment was maintained at 30°C, apparently lower than the lethal temperature solarization required, which was usually in the range of 45–55°C (Katan, 2000; Larkin and Griffin, 2007; Yossen et al., 2008). Also, flooding was not significantly contributed to pathogen inactivation, since the most dramatic reduce of viable FOC was observed in the soils amended with crop straws. The introduction of organic amendments created strong anaerobic and reductive soil conditions, which greatly influenced the soil ecosystem and finally led to pathogen inactivation. The whole process is complex, involving changes in soil physical and chemical properties, shifts in microbiology community from aerobic-dominated microbiology community to anaerobic-dominated microbiology community, and release and accumulation of volatile compounds or organic acids (Butler et al., 2012b; Gamliel et al., 2000; Kobara et al., 2007; Lazarovits et al., 2001; Momma et al., 2013; Tenuta and Lazarovits, 2002; Mowlick et al., 2014). Momma et al. (2011, 2013) noted that the process was usually accompanied with a kind of intense peculiar odor, which also emanated in our experiment 3 days after incubation. The detailed mechanism is needed further research to show evidence of our assumption.

4. Conclusions

Biological soil disinfestation, *i.e.*, coupled incorporation of decomposable organic materials with flooding or water saturation to create strong reductive and anaerobic conditions, is an effective approach for control soil-borne diseases. Our experiment demonstrated that flooding alone could suppress the population of FOC, typical banana wilt pathogens, but was not as effective as coupled with either rice straw or maize straw. The effectiveness on FOC inactivation was not different significantly between rice straw and maize straw, between the application rates of 1.5 and 3.0 tons/ha, and between under flooded and water saturated conditions. The population of FOC in the soils

amended with crop straw decreased sharply in the first 15-day after incubation under flooded or water saturated conditions and then fluctuated. Therefore, coupled incorporation of either rice straw or maize straw with flooding or water saturation for 15 days would be an effective management strategy for controlling banana wilt pathogens. However, further evaluations in field conditions are essential to develop a practical and economical approach to control banana *Fusarium* wilt. In the meantime, the impact of the method on root growth, banana yield and market value has yet to be demonstrated.

Acknowledgment

This work was supported by the National Natural Science Foundation of China (Nos. 41222005, 41330744, 413301335), the Natural Science Foundation of Jiangsu Province (Nos. BK2010611, SBK201220477), Research Fund of State Key Laboratory of Soil and Sustainable Agriculture, Institute of Soil Science, Chinese Academy of Sciences (Y412201404) and the Priority Academic Program Development of Jiangsu Higher Education Institutions. We sincerely thank colleagues from Hainan University for their kindly help in the field and laboratory work. We also thank colleagues from Hainan Wan Chung Industrial Co., Ltd. for their warm assistance in field survey. The authors are very grateful to the editors and reviewers for their invaluable comments and suggestions.

REFERENCES

Alabouvette, C., Olivain, C., Migheli, Q., Steinberg, C., 2009. Microbiological control of soil-borne phytopathogenic fungi with special emphasis on wilt-inducing *Fusarium oxysporum*. New Phytol. 184 (3), 529–544.

Blok, W.J., Lamers, J.G., Termorshuizen, A.J., Bollen, G.J., 2000. Control of soilborne plant pathogens by incorporating fresh organic amendments followed by tarping. Phytopathology 90 (3), 253–259.

Buresh, R., Sayre, K., 2007. Implications of straw removal on soil fertility and sustainability. Expert Consultation on Biofuels. IRRI, Los Banos, Philippines, p. 34.

Butler, D.M., Kokalis-Burelle, N., Muramoto, J., Shennan, C., McCollum, T.G., Rosskopf, E.N., 2012a. Impact of anaerobic soil disinfestation combined with soil solarization on plant-parasitic nematodes and introduced inoculum of soilborne plant pathogens in raised-bed vegetable production. Crop. Prot. 39, 33–40.

Butler, D.M., Rosskopf, E.N., Kokalis-Burelle, N., Albano, J.P., Muramoto, J., Shennan, C., 2012b. Exploring warm-season cover crops as carbon sources for anaerobic soil disinfestation (ASD). Plant Soil 355 (1–2), 149–165.

Dita, M.A., Waalwijk, C., Buddenhagen, I.W., Souza Jr., M.T., Kema, G.H.J., 2010. A molecular diagnostic for tropical race 4 of the banana *Fusarium* wilt pathogen. Plant Pathol. 59 (2), 348–357.

Gadde, B., Bonnet, S., Menke, C., Garivait, S., 2009. Air pollutant emissions from rice straw open field burning in India, Thailand and the Philippines. Environ. Pollut. 157 (5), 1554–1558.

Gamliel, A., Austerweil, M., Kritzman, G., 2000. Non-chemical approach to soilborne pest management—organic amendments. Crop. Prot. 19 (8–10), 847–853.

Helslop-Harrison, J.S., Schwarzacher, T., 2007. Domestication, genomics and the future for banana. Ann. Bot. 100 (5), 1073–1084.

Hrynchuk, L., 1998. Rice Straw Diversion Plan. California Air Resources Board, Sacramento, CA.

Katan, J., 2000. Physical and cultural methods for the management of soil-borne pathogens. Crop. Prot. 19 (8–10), 725–731.

Klein, E., Katan, J., Gamliel, A., 2011. Combining residues of herb crops with soil heating for control of soilborne pathogens in a controlled laboratory system. Crop. Prot. 30 (3), 368–374.

Klein, E., Katan, J., Gamliel, A., 2012. Soil suppressiveness to *Meloidogyne javanica* as induced by organic amendments and solarization in greenhouse crops. Crop. Prot. 39, 26–32.

Kobara, Y., Uematsu, S., Tanaka-Miwa, C., Sato, R., Sato, M., 2007. Possibility of the new soil fumigation technique with ethanol solution. Proceedings of the 2007 Annual International Research Conference on Methyl Bromide Alternatives and Emissions Reductions, p. 74.

Komada, H., 1975. Development of a selective medium for quantitative isolation of *Fusarium oxysporum* from natural soil. Rev. Plant Prot. Res. 8, 114–124.

Larkin, R.P., Griffin, T.S., 2007. Control of soilborne potato diseases using *Brassica* green manures. Crop. Prot. 26 (7), 1067–1077.

Lazarovits, G., Tenuta, M., Conn, K.L., 2001. Organic amendments as a disease control strategy for soilborne diseases of high-value agricultural crops. Australas. Plant Pathol. 30 (2), 111–117.

Lievens, B., Brouwer, M., Vanachter, A.C., Levesque, A., Cammue, B., Thomma, B., 2005. Quantitative assessment of phytopathogenic fungi in various substrates using a DNA macroarray. Environ. Microbiol. 7 (11), 1698–1710.

Lievens, B., Brouwer, M., Vanachter, A.C., Cammue, B., Thomma, B.P., 2006. Real-time PCR for detection and quantification of fungal and oomycete tomato pathogens in plant and soil samples. Plant Sci. 171 (1), 155–165.

Messiha, N.A., van Diepeningen, A.D., Wenneker, M., van Beuningen, A.R., Janse, J.D., Coenen, T.G., et al., 2007. Biological soil disinfestation (BSD), a new control method for potato brown rot, caused by *Ralstonia solanacearum* race 3 biovar 2. Eur. J. Plant Pathol. 117 (4), 403–415.

Mitchell, R., Alexander, M., 1962. Microbiological changes in flooded soils. Soil Sci. 93 (6), 413–419.

Momma, N., 2008. Biological soil disinfestation (BSD) of soilborne pathogens and its possible mechanisms. Jpn. Agric. Res. Q. 42 (1), 7–12.

Momma, N., Yamamoto, K., Simandi, P., Shishido, M., 2006. Role of organic acids in the mechanisms of biological soil disinfestation (BSD). J. Gen. Plant Pathol. 72 (4), 247–252.

Momma, N., Momma, M., Kobara, Y., 2010. Biological soil disinfestation using ethanol: effect on *Fusarium oxysporum* f. sp. *lycopersici* and soil microorganisms. J. Gen. Plant Pathol. 76 (5), 336–344.

Momma, N., Kobara, Y., Momma, M., 2011. Fe^{2+} and Mn^{2+}, potential agents to induce suppression of *Fusarium oxysporum* for biological soil disinfestation. J. Gen. Plant Pathol. 77 (6), 331–335.

Momma, N., Kobara, Y., Uematsu, S., Kita, N., Shinmura, A., 2013. Development of biological soil disinfestations in Japan. Appl. Microbiol. Biotechnol. 97 (9), 3801–3809.

Mowlick, S., Takehara, T., Kaku, N., Ueki, K., Ueki, A., 2013. Proliferation of diversified clostridial species during biological soil disinfestation incorporated with plant biomass under various conditions. Appl. Microbiol. Biotechnol. 97 (18), 8365–8379.

Mowlick, S., Inoue, T., Takehara, T., Tonouchi, A., Kaku, N., Ueki, K., et al., 2014. Usefulness of Japanese-radish residue in biological soil disinfestation to suppress spinach wilt disease accompanying with proliferation of soil bacteria in the *Firmicutes*. Crop. Prot. 61, 64–73.

Muramoto, J., Shennan, C., Fitzgerald, A., Koike, S., Bolda, M., Daugovish, O., et al., 2008. Effect of anaerobic soil disinfestation on weed seed germination. Proceedings of the Annual International Research Conference on Methyl Bromide Alternatives and Emissions Reductions. Orlando, USA. Nov 10–14.

Núñez-Zofío, M., Larregla, S., Garbisu, C., 2011. Application of organic amendments followed by soil plastic mulching reduces the incidence of *Phytophthora capsici* in pepper crops under temperate climate. Crop. Prot. 30 (12), 1563–1572.

O'Donnell, K., Kistler, H.C., Cigelnik, E., Ploetz, R.C., 1998. Multiple evolutionary origins of the fungus causing Panama disease of banana: concordant evidence from nuclear and mitochondrial gene genealogies. Proc. Natl. Acad. Sci. U. S. A. 95 (5), 2044–2049.

Ploetz, R.C., 2006. *Fusarium* wilt of banana is caused by several pathogens referred to as *Fusarium oxysporum* f. sp. *cubense*. Phytopathology 96 (6), 653–656.

Shennan, C., Muramoto, J., Bolda, M., Koike, S.T., Daugovish, O., Rosskopf, E., et al., 2007. Optimizing anaerobic soil disinfestation: an alternative to soil fumigation? Proceedings of the Annual International Research Conference on Methyl Bromide Alternatives and Emissions Reductions. San Diego, USA. Oct 29–Nov 1

Shinmura, A., 2002. Studies on the ecology and control of Welsh onion root rot caused by *Fusarium redolens*. J. Gen. Plant Pathol. 68 (3), 265-265.

Snyder, G.H., 1987. Agricultural flooding of organic soils. Bulletin/University of Florida. Agric. Exp. Station 41–56.

Snyder, W.C., Hansen, H., 1940. The species concept in *Fusarium*. Am. J. Bot. 27 (2), 64–67.

Stover, R., Ploetz, R., 1990. *Fusarium* wilt of banana: some history and current status of the disease. *Fusarium* Wilt of Banana. APS Press, pp. 1–7.

Subbarao, K.V., Hubbard, J.C., Koike, S.T., 1999. Evaluation of broccoli residue incorporation into field soil for *Verticillium* wilt control in cauliflower. Plant Dis. 83 (2), 124–129.

Tenuta, M., Lazarovits, G., 2002. Ammonia and nitrous acid from nitrogenous amendments kill the microsclerotia of *Verticillium dahliae*. Phytopathology 92 (3), 255–264.

Wang, B.B., Yuan, J., Zhang, J., Shen, Z.Z., Zhang, M.X., Li, R., et al., 2013. Effects of novel bioorganic fertilizer produced by *Bacillus amyloliquefaciens* W19 on antagonism of *Fusarium* wilt of banana. Biol. Fertil. Soils 49 (4), 435–446.

Yossen, V., Zumelzu, G., Gasoni, L., Kobayashi, K., 2008. Effect of soil reductive sterilisation on *Fusarium* wilt in greenhouse carnation in Córdoba, Argentina. Australas. Plant Pathol. 37 (5), 520–522.

Zhang, R., Zhang, Z., 1999. Biogasification of rice straw with an anaerobic-phased solids digester system. Bioresour. Technol. 68 (3), 235–245.

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Journal of Environmental Sciences (Established in 1989)
Volume 31 2015

Supervised by	Chinese Academy of Sciences	**Published by**	Science Press, Beijing, China
Sponsored by	Research Center for Eco-Environmental		Elsevier Limited, The Netherlands
	Sciences, Chinese Academy of Sciences	**Distributed by**	
Edited by	Editorial Office of Journal of	Domestic	Science Press, 16 Donghuangchenggen
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Editor-in-chief	X. Chris Le	**Printed by**	Beijing Beilin Printing House, 100083, China

CN 11-2629/X **Domestic postcode: 2-580** **Domestic price per issue RMB ¥ 110.00**

ISSN 1001-0742