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A Biosecure Composting System for *Tilletia controversa Kühn*-Infected Wheat Waste*

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ABSTRACT

Tilletia controversa Kühn (TCK) has strong infectivity and viability and may cause great cut hazard to wheat and other crops. Composting treatment of TCK-infected wheat waste may be an effective method to eliminate the further contamination. This study applied microbial fermentation composting technique using mixed crop straws and manure to establish a bio-friendly composting method. The change of physicochemical properties of the compost was monitored regularly to detect the time course of TCK degradation and confirm the inactivation of TCK germination activity. The results of regular sampling indicate that the germination rate of TCK declines with the composting progress, and composting ten days under the condition of 50˚C to 60˚C completely inactivates the germination ability of TCK. Thus the present study provides a bio-friendly composting method for degrading TCK-infected wheat crops under TCK outbreak.

Keywords: Composting; *Tilletia controversa Kühn*; Degradation

1. Introduction

Tilletia contraversa Kühn (commonly abbreviated TCK) belongs to Fungi, Basidiomycotina, Teliomycetes, Ustilaginales, Tilletiaceae, *Tilletia*. TCK is a plant quarantine fungus that is greatly harmful and causes 20% to 75% reduction in wheat production. In the epidemic outbreak years, it can eventually result in no harvest at all [1]. TCK grows up with the wheat together and it invades wheat inflorescence at booting stage. Finally, TCK forms mycocecidium which replaces the whole kernel. The shape of mycocecidium is similar to a ball and the surface of mycocecidium is highly tough. Mycocecidium is full of black teleutospore which owns a stronger survival ability. Teleutospore is primary infection resources and it will germinate at least three weeks if the temperature (3˚C - 8˚C), sunlight and humidity are suitable. The viability of TCK fungus galls scattered in the field lasts up to 10 years, and the germination rate of TCK teliospores gone through animal's digestive tract can be as high as 64% [2]. If the TCK-infected straws, wheat bran, and other wastes remain active, once the infectious source has formed, or the fungus has accumulated in the fields to a relatively high density in an area under years of nonstop crop rotation, TCK diseases will become more and more severe. Moreover, in the annual harvest season, the thick smoke-generating straw burning not only greatly harms the environment, but also wastes the straw resources. The inspection and quarantine management department in China requires that the storage and treatment of waste materials generated during the grain process should be centralized, to prevent the potential spreading of pests. Therefore, treating TCK-infected straws, wheat bran, and other wastes, in a biosecure way to transform them into usable products, becomes the best approach to both block the epidemics and control the hazard. This study has applied microbial fermentation-based composting techniques to inactivate TCK in wheat wastes via fermentation and composting maturation with mixed crop straws and manures.

2. Materials and Methods

2.1. The Tested Fungal Strain and Culture Medium

The TCK fungus galls collected in 2009 were provided by the Xinjiang Entry-Exit Inspection and Quarantine Bureau of China. The water agar (WA) medium con-

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tained 30 g of agar and 1 L of distilled water.

2.2. The Instruments

Autoclave, superclean bench, low temperature incubator, microscope, temperature sensor, pH meter, elemental analyzer, freeze-drying machine, and compost bin (dimension: 280 cm × 120 cm × 100 cm; thickness: 10 cm) were used in this study.

2.3. The Establishment of Composting System

The cow dungs were collected freshly from a cattle farm in Dalian city of China and made homogeneous by thorough mix. The C/N ratio of the compost was adjusted to 19:1 with straws, and its water content was adjusted to 63.7% by drying. The composting materials were piled into the bottomless compost bin to start the static composting. The compost bin was loosely covered with lid to not only provide a shelter but also allow the gas exchange between the pile and the environment. A pad of 20 cm thick paddy straw was placed on the bottom of compost bin to absorb the exudate. Under sterile condition, the intact TCK gall was placed into 1.5 mL sterilized tube, which was then buried 40 - 50 cm deep inside the compost. Multiple thermocouples were also placed close to the sample tube, and the compost temperature was measured daily. After 10 days' composting fermentation, compost was required to turn over to make sure the mixture of various ingredients in composting system.

2.4. The Detection of Physicochemical Parameters

The TCK gall samples were randomly collected from multiple sites in the compost after 0, 2, 4, 6, 10, 14, 18, 26, 32, and 40 days of composting, respectively, to detect the pH value, moisture, and C/N ratio. Briefly, 2 g of compost sample were weighed, put into a 50 mL test tube, added with 18 mL of distilled water, and placed on a Vortex mixer to shake for 5 min and stay static for another 30 min. The pH value of the supernatant was then measured using a pH meter. The moisture was determined by freeze-drying method. The freeze-dried compost sample was ground evenly and used to detect the C/N ratio.

2.5. The Germination Rate of Composting-Treated TCK

The germination rate of treated TCK were detected after 2, 4, 6, 10, 14, 18, 26, 32, and 40 days of composting, respectively, and compared with that in control group. Briefly, the teliospores removed from the fungal gall samples were suspended in appropriate amount of sterile water and spread evenly onto WA plate with approximately 50 teliospores per field. The plate was then placed at 4˚C and cultured under continuous light condition. The observation was started 3 weeks later. The germination rate was calculated 6, 8, and 12 weeks, respectively, after the cultivation. The average germination rate was calculated from triplicated tests. The length of promycelium greater than or equal to the diameter of TCK teliospore was recognized as germinating.

3. Results

3.1. The Changes in Compost Temperature

The temperature is an important factor affecting the microbial activities and the composting processes. High temperature contributes to the rapid inactivation of the pathogens, and an appropriate temperature range is conducive to humus formation. As shown in **Figure 1**, 2 - 8 days after the composting, the temperature was maintained between 50˚C - 60˚C, without influence from the ambient temperature. In the 5th day of composting, the temperature reached the maximum approximately 55˚C. In the 10th day, the temperature dropped to approximately 30˚C, and the compost was mixed again. The temperature of mixed compost decreased initially, then began to rise, and finally dropped to room temperature after 14 days of maintaining between 40˚C - 50˚C.

3.2. The Changes of pH in the Compost

Figure 2 shows that in the beginning, the pH of the composting materials increases rapidly. After reaching 8.9 at the 2nd day, the pH began to decline slowly until the 10th day. The pH values were between 8.5 and 8.9. After the 10th day's mixing, until the 40th day, the compost pH maintained in the range of 8.5 to 9.1. Microorganisms survive within certain pH range; a stable, suitable pH facilitates the microbial fermentation and accelerates the composting process.

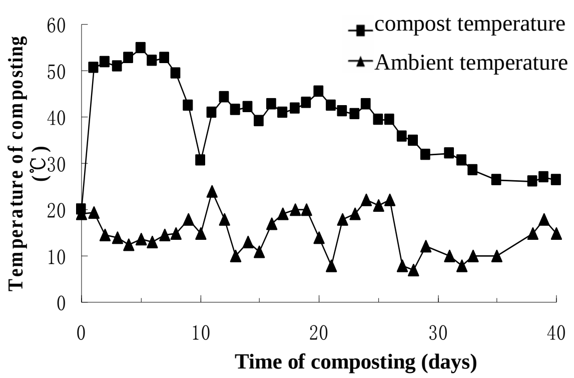

Figure 1. Changes of temperature in compost during static composting period.

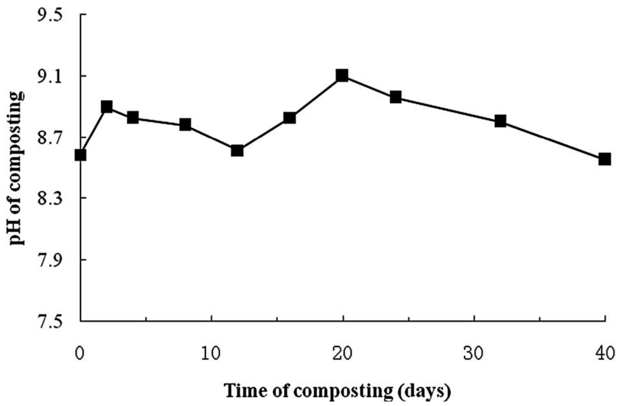

Figure 2. Changes of pH in compost during static composting period.

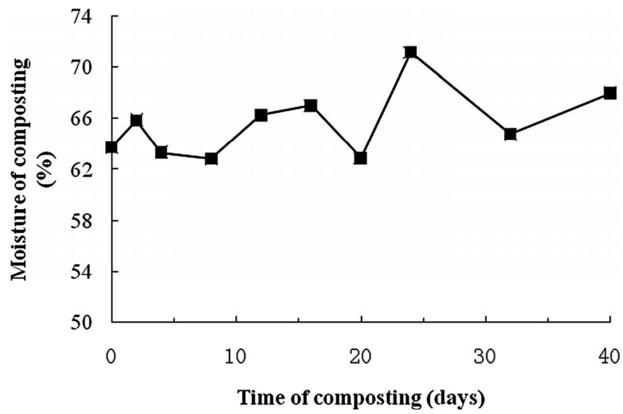

Figure 3. Changes of t moisture in compost during static composting period.

3.3. The Changes of Moisture in the Compost

During the composting process, microorganisms can only absorb the soluble organic matters; therefore, the compost should contain a certain amount of moisture. Overly low moisture slows down or eventually stops the microbial decomposition process and may also lead to an uneven distribution of temperature in the compost. Conversely, high moisture results in insufficient oxygen supply, leading to an anaerobic environment, which affects the metabolism of microorganisms. **Figure 3** indicates that the initial moisture content was 63.7%, which increased to 66% in the 2nd day and maintained between 60% and 71% thereafter until the 40th day, indicating that the compost is suitable for the growth of microorganisms, which facilitates the microbial degradation of cellulose, lignin, etc., and promotes the fertilizer transformation.

3.4. The C:N Ratio in the Compost

As shown in **Figure 4**, the initial C:N ratio in the compost was 19.1. In the 2nd day, the C:N ratio significantly decreased, but became relatively stable thereafter and maintained at approximately 11.0. Morel *et al.* [3]. Suggest that use T = (end point C:N ratio)/(initial C:N ratio) to evaluate the maturity of refuse compost, and a stable T < 0.6 indicates the compost maturity. After calculation, at the end of this study, the compost T value is 0.58, indicating that after 40 days of composting, the compost reaches maturity and can be further processed as fertilizer.

3.5. The Inactivation of TCK Determined by Germination Rate

The germination activity of TCK sample was tested as described in the materials and methods. The germination rates were observed and calculated in the 6th, 8th, and 12th week of cultivation, respectively. As shown in **Table 1**, almost all the TCK in control group germinated

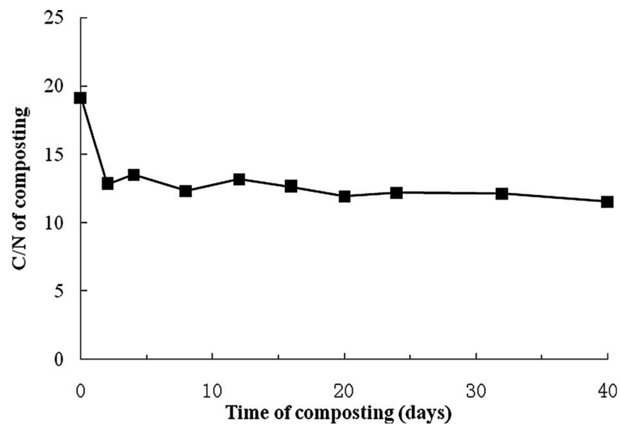

Figure 4. Changes of C:N ratio in compost during static composting period.

Table 1. The effect of composting time on the germination rates of TCK.

Sampling time (d)	The germination rate of TCK after different time of culture		
	6 weeks	8 weeks	12 weeks
Control	69.75%	89.35%	ND*
2	15.90%	22.25%	30.67%
4	0	5.12%	7.28%
6	0	9.34%	15.48%
10 - 40	0	0	0

*ND means not done.

after culture for more than 8 weeks, indicating a very high germinating activity and strong infectivity of the experimental TCK. The germination rate of TCK started to decline in the 2nd day of composting; when cultured for 6 weeks, only a small amount of them germinated; after 12 weeks of culture, 30.67% of the teliospores survived. After composting for 4 and 6 days, there was no germination of TCK in 6 weeks after the cultivation, and only part of the TCK germinated after culture for 8 and

12 weeks (**Figure 5**). After composting for 10, 14, 18, 26, 32, and 40 days, there were no germinations of the sampled TCK, indicating that the TCK had been completely inactivated (**Figure 6**).

4. Discussion

Irradiation [4-6], drug fumigation [7,8], and heat sterilization [9] are current common methods used to treat TCK-infected produce. However, the irradiation method has not been widely used due to the limited penetration capability of the electron beam and the influence of device flexibility. Drug fumigation causes certain damages to the environment, which hinders its practical applications. Heat sterilization reduces the wheat quality. Therefore, the above three methods encounter certain difficulties in treating wheat products that carry large amount of TCK. The composting takes advantage of microorganisms transforming unstable organic matters into stable humus, and the heat generated in the composting process is able to kill the pathogens, weed seeds, eggs, etc. In addition, the soil microbes in the compost can degrade the cellulose, lignin, and other important plant components, and the transformed products can be used as fertilizers. Such treatment can protect the environment, improve the soil fertility, and increase the soil water holding capacity, as well as reduce the volume of organic materials, odor, and pathogens, to achieve the recycling of resources [10]. Although researchers have already used composting techniques to process and inactivate the bacteria- and viruses-infected animals [11-14], plants [15, 16], and genetically modified organisms [17], there is a lack of study regarding the composting inactivation of fungal TCK.

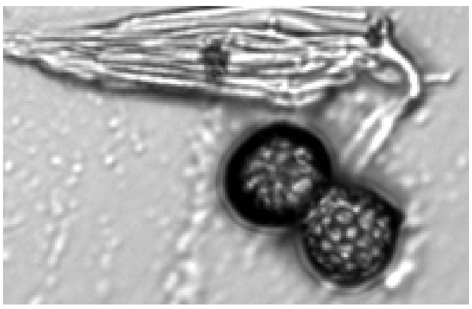

Figure 5. TCK teleutospore germinated and formed to be basidiospore after cultivation.

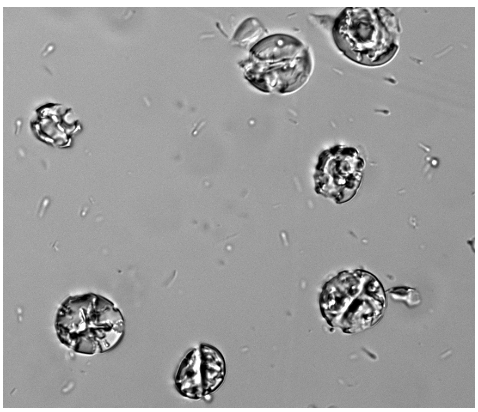

Figure 6. TCK teleutospore didn't germinated after cultivation.

In this study, aerobic microorganisms divide vigorously in the early phase of composting, and the easy-to-decompose organic matters are degraded firstly. The released heat increases the compost temperature to 55˚C in 5 days. As the composting progresses, due to the microbial consumption of large amounts of carbohydrates, the total C exhibits a declining trend, while the consumption of N is relatively slow, resulting in a reduced C:N ratio. The decomposition products contain large quantities of ammonia and water; the ammonia dissolves in the liquid phase, leading to a rapid increase in pH. When the compost temperature reaches its peak, and the oxygen supply is relatively sufficient, the thermophilic bacteria begin to break down complex organic compounds, maintaining the compost temperature in a certain range. The effects of appropriate temperature, moisture, pH, microbial fermentation, as well as other factors, finally inactivate the TCK.

In conclusion, the present study has shown that TCK completely loses its bioactivity after 10 days of composting under 50˚C - 60˚C, indicating that bio-friendly composting method established in this study has the ability to inactivate TCK-infected wheat crops under the outbreak of TCK.

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