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# IMPROVEMENT IN CONTINUOUS CROPPING OF CUT CHRYSANTHEMUM BY *PHANEROCHAETE CHRYSOSPORIUM*

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**Abstract**

Cut chrysanthemum is one of the most popular cut flowers in the world. However, its production tends to fall dramatically because of continuous cropping. This reduced production may be due to the accumulation of phenolic acids secreted by the plant root and deposited in the soil. In this study, the chlamydospore of *Phanerochaete chrysosporium* was used to test the degradation ability of four phenolic acids after being inoculated into *C. morifolium* rhizosphere that has been monocultured for 5 years. The degradation rate of ferulic acid, syringic acid, vanillic acid, and *p*-hydroxybenzoic acid increased to 94.0%, 78.8%, 84.2%, and 81.4%, respectively, in comparison with CK. At the same time, urease content in the soil was augmented, which could effectively help in stimulating the nitrogen cycle. The fungi amount in the treatment group decreased in comparison with CK, whereas the bacteria content in the treatment group increased evidently by 3.00 and 1.76 times the control group content. The physiological status of plants improved after 120 days of cultivation, thereby illustrating that *P. chrysosporium* exerts a positive effect on addressing the issue of continuous cropping soil.

**Key words:** Cut chrysanthemum, *Phanerochaete chrysosporium*, Chlamydospore, Continuous cropping, Phenolic acids, Urease.

## Introduction

*Chrysanthemum morifolium* (Chrysanthemum), which belongs to the genus *Chrysanthemum*, is a commercially important flowering shrub that is native to Asia and northeastern Europe. The shrub was first cultivated in China as far back as fifteenth century B.C. It ranks second after the rose in dominance in the global cut flower market (Spaargaren, 2002). With the rapid economic development in the 1980s, the requirement for *C. morifolium* increased on the basis of its utilization in four aspects: (a) tea use, (b) medicinal use, (c) ornamental use, and (d) edible use. Therefore, the cultivation area for *C. morifolium* has expanded, and its output is increasing rapidly. In 2005, national export of *C. morifolium* reached 37 million, and *C. morifolium* has become a vital economic crop in the country. However, during long periods of cultivation, the continuous cropping system exerts a negative effect on soil conditions, thereby reducing *C. morifolium* productivity. Plants were seriously reduced after continuous cropping, and negative physiological characteristics, such as dwarfism or stunted growth, dead leaves, inferior quality, and low quantity, were observed. Over time, the plants would suffer complete loss from disease and insect pests during harvest. This type of long-term cropping soil is highly typical in China and other countries. Soybean yield was seriously affected by the cyst nematode and soil-borne diseases in continuous systems (Liu and Yu, 2000; Xu *et al.*, 2000). Asparagus yield and quality decline are due to natural aging after planting (Yasufumi *et al.*, 2012). More than 90% coverage of cotton yield has decreased substantially because of the single crop species in the area (Zhang *et al.*, 2013). The damage in numerous crops, vegetables, and fruits necessitates an understanding of the mechanism of long-term cropping, and effective approaches to overcome this problem should be found. A previous study found that autotoxicity, which is one of the obvious characteristics of allelopathy, especially for root exudates (phenolic acids), may influence the germination and growth of plants (Cao *et al.*, 2001). Allelopathy is a biological phenomenon by which an organism produces one or more biochemicals that influence the growth, survival, and reproduction of other organisms. The tissues of numerous plants secrete large amounts of *p*-hydroxybenzoic acid, vanillic acid, syringic acid, and ferulic acid, which could irreversibly damage root growth, decrease leaf chlorophyll, and degrade soil fertility. This effect was the main explanation for the continuous cropping obstacle (Olk *et al.,* 2009; Huang *et al.,* 2010; Chen *et al.*, 2011).

Biological control method is less polluting and has therefore gradually replaced traditional chemical pesticides. Certain types of bacteria and fungi perform vital functions in the soil ecosystem and act as natural antagonists of plant pathogens and decomposers of plant residues (Gardener & Fravel, 2002; Gao *et al.*, 2012; Ahmad *et al.,* 2014; Shafique *et al.,* 2015). *Phanerochaete chrysosporium* is a typical representative of the white rot fungus that belongs to Thelephoraceae. The chlamydospore is a large, thick-walled resting spore that exhibits better stress resistance than conidia and can therefore preserve, germinate, and survive easily (Jiménez-Tobon *et al.*, 2003; Chung *et al.*, 2005). Most previous studies focused on the function of the fungus in the biodegradation of lignin because of its strong extracellular oxidative enzyme system and related compounds found in explosive contaminated pesticides, materials, and toxic wastes. Owing to the advantages of degrading phenolic acids deposited in the soil and increasing microbial diversity and abundance, the conidia of *P. chrysosporium* has been used to overcome the continuous cropping barrier of cucumber (Xu *et al.*, 2008). Studies on long-term cropping of *C. morifolium* are rare. In this research, the chlamydospore of *P. chrysosporium* was inoculated into *C. morifolium* rhizosphere as a biological control method for use in long-term cropping of *C. morifolium* field. This study is the first to monitor the effect of *P. chrysosporium* on changes in microbial communities, phenolic acids, and enzyme activity in *C. morifolium* rhizosphere.

## Materials and Methods

**Strain and chlamydospore preparation:** *Phanerochaete* sp. HSD, a strain of white rot fungus, was isolated from a tree full of rot from the pinehurst of Taihang Mountain in Henan Province, China. The strain was maintained on potato dextrose agar slant at 4°C and sub-cultured every 3 months. Chlamydospores were prepared according to the method of Wang (2009).

**Experimental site and description:** The experimental site was located in a Weiyuan plantation field in Xinxiang, Henan Province, China (35.17° N, 113.59° E), in which the quality of *C. morifolium* was seriously affected by fusarium wilt during long-term cropping and the yield has not reached 10% of that in the first year. The planting plot in this field consisted of 16 rows with a 0.5 m interval. Ten and twenty-five grams of cultivated chlamydospore were sprayed on the surface of the soil one day before *C. morifolium* planting and re-sprayed one month later with the same dosage after decapitation. T20 and T50 were marked according to the common dosage that was used. Unsprayed rows were treated as the control check group (CK).

**Sampling:** At each time point, 10 plants were selected randomly from every row, and physiological indices, including plant height, stem diameter, leaf length and width, and chlorophyll content, were documented at 30, 60, 90, and 120 days, respectively. The third leaf from the plant apex was used to determine chlorophyll content with the use of a SPAD-502 chlorophyll meter. Soil samples were taken from 5 cm to 10 cm below the surface and 5 cm away from the plants, and were collected from each treatment after every 15 days. A minimum of 5 samples were taken from each plot at each time, and biochemical indices, such as enzyme and phenolic acid contents, were documented.

**Determination of phenolic acids:** Using the method of Heimler and Pieroni (1994), 10 g of soil were stirred in 50 mL water for 24 h. After centrifugation and filtration, aqueous solutions were used for bioassay, and 10 mL of the solutions were acidified with 2 M HCl to pH 2.8. Then, humic acid was removed under 2000 r/min centrifugation. Next, 2 mL of the organic phase was concentrated to dryness on a rotary evaporator (50°C) and used for high-performance liquid chromatography (HPLC). The standard compounds of *p*-hydroxybenzoic acid, vanillic acid, syringic acid, and ferulic acid were prepared before use. A 0.5 g ($1.0\times10^7$ cfu/g) of *P. chrysosporium* chlamydospore was inoculated in 300 mL of the infusion (2.0 g/mL) that was prepared in the previous step. The content of phenolic acids in both inoculated and blank control was measured after 7 days of cultivation by using HPLC.

**Enzyme activity assay:** The enzyme activity of catalase was measured by using ultraviolet spectrophotometry (Chance and Maehly, 1955), whereas urease was measured by Berthelot colorimetric method (Greno *et al*., 1970).

**Quantity of soil microorganisms:** All soil samples were taken at 5 cm to 10 cm below the surface and 5 cm away from the plants. Ten grams of each soil sample was mixed with 90 mL sterile water and then diluted to different concentration gradients. Three replicates were prepared for each concentration. Bacterial and fungal quantity were calculated by using traditional cultivation method, and 100 μL of the diluents was cultivated on a suitable culture medium at the corresponding time and temperature (bacteria were cultured on peptone beef medium for 1 day at 37°C, fungi were cultured on Martin medium, and *Actinomycetes* were cultured on improved Gause 1 cultural medium for 2 days to 3 days at 28°C).

## Results

**Influence of *P. chrysosporium* on phenolic acids:** Microbial activity is considerably influenced by the root because of exudation of certain sugars, organic acids, and amino acids into the soil. From 0 days to 75 days, the total content of phenolic acids in both CK and treatment groups increased dramatically in comparison with the original level. From 75 days to 120 days, the total content of phenolic acids in treatment groups T20 and T50 was markedly reduced in contrast to that of CK, thereby indicating that inoculated *P. chrysosporium* had an effect on these allelochemicals (Fig. 1).

The degradation ability of *P. chrysosporium* on four different phenolic acids after 72 h of processing increased significantly (Fig. 2). The degradation rate of ferulic acid, syringic acid, vanillic acid, and *p*-hydroxybenzoic acid reached up to 94.0%, 78.8%, 84.2%, and 81.4%, respectively, in comparison with CK.

**Change of enzyme activity in the rhizosphere:** Urease could catalyze the hydrolysis of urea, which cannot directly be used by the plant as nitrogen source into the readily utilizable ammonia (Chelikani *et al*., 2004). Catalase could catalyze the decomposition of hydrogen peroxide to water and oxygen, thereby protecting the cell from oxidative damage and reducing the poison from hydrogen peroxide on the plant.

In comparison with CK, the catalase content in both T20 and T50 changed slightly in the entire process and exhibited a minimal increase in the final stage (Fig. 3A). Meanwhile, marked changes were observed in urease content in the comparison of the treatment group with CK, and the urease content in T20 and T50 was higher than that of CK in the most cultivated stage (Fig. 3B).

**Change in microbial community:** Previous research demonstrated that the complex physico-chemical characteristics of soil affect plant physiology and root exudation, thereby subsequently influencing microbiota composition (Tang *et al*., 2009; Philippot *et al*., 2013). At the same time, the microorganism exerts profound effects on the growth nutrition and health of plants. The structure of the microbial community in rhizospheres could change during different life stages of plants. Variations in bacterial and fungi were reportedly observed in rhizosphere soils at several vegetative and reproductive stages of *Medicago truncatula* Gaertn (Mougel *et al*., 2006).

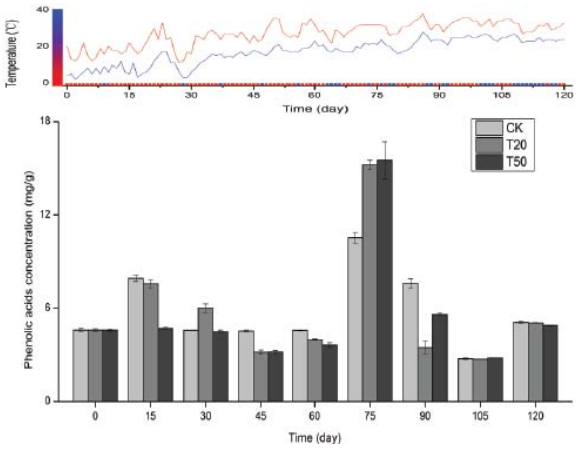


Fig. 1. Change in total phenolic acid concentration from 0 day to 120 days of cultivation after inoculation of *P. chrysosporium*. CK: control check group; T20, T50: treatment groups.

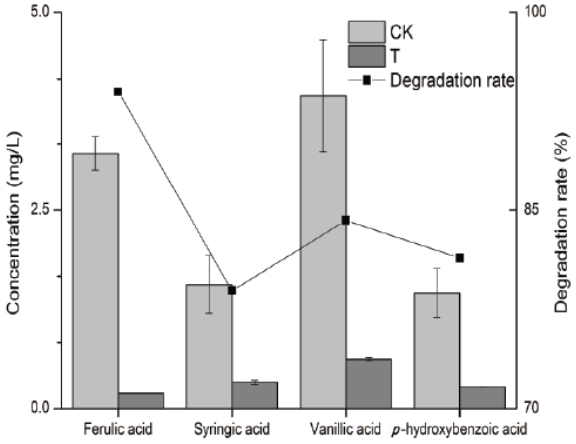


Fig. 2. Degradation ability of *P. chrysosporium* on four different phenolic acids. CK: control check group; T: treatment group.

The trend of bacterial change in treatment and CK groups was consistent and showed that bacterial amount fluctuated along with cultivation time. In most stages, bacterial content examined in T20 and T50 exceeded that of CK. Then, after 120 days of cultivation, the bacterial amount in T20 and T50 was 1.47 and 1.65 times that in the control group, respectively (Fig. 4A). The results illustrate that *P. chrysosporium* could promote bacterial growth in soil. The total amount of fungi was extremely low in the initial processing stage in both treatment and CK groups, and the total soil culturable fungi was approximately $21\times10^1$ cfu/g dried soils. After 90 days of processing, mass fungi multiplication occurred in each group, and the amounts of fungi in T20 and T50 were considerably lower than that in CK (Fig. 4B). Results show that *P. chrysosporium* could slow down the propagation of fungi.

**Improvement in physiological status of plants:** At 120 days of harvest time, the growth condition of *C. morifolium* in the treatment groups was superior to that in CK. Plant height in T20 reached 71.32 cm, which is a 21.67% increase over that in CK with a low incidence rate (Table 1). At the same time, the stem diameter in T20 and T50 increased by 21.43% and 16.07%, respectively, in comparison with CK. In addition, the chlorophyll content in T20 and T50 exceeded that of CK. These findings indicate that the physiological characteristics of continuously cropped *C. morifolium* improved through inoculation of *P. chrysosporium*.

## Discussion

**Degradation ability of *P. chrysosporium* on phenolic acids:** The increasing content of phenolic acids from 0 days to 75 days in both CK and treatment groups indicate the accumulation of specific plant root exudates in soil. The reduced content of phenolic acids in treatment groups T20 and T50 in contrast to that of CK from 75 days to 120 days demonstrated that the inoculation of *P. chrysosporium* could effectively degrade phenolic acids that have accumulated in the soil and alleviate self-allelopathy. Vanillic acid is a dihydroxybenzoic acid derivative and is an oxidized form of vanillin. The secretion of vanillin acid by plant roots could promote the growth and development of *Fusarium oxysporum* f.sp. *niveum*, thereby resulting in serious fusarium wilt (Wu *et al.*, 2008). After continuous cropping, *C. morifolium* was seriously affected by fusarium wilt. Therefore, decreasing the content of vanillic acid by *P. chrysosporium* could inhibit *F. oxysporum* growth effectively and reduce growth obstacles. The inhibitive effect of ferulic acid, *p*-hydroxybenzoic acid, and syringic acid on plant seedling growth was verified (Zhou *et al.*, 2012; 2014), and decreasing their accumulation could promote plant growth. The above results demonstrated that *P. chrysosporium* showed high efficiency in degrading these phenolic acids and could reduce the continuous cropping obstacle.

**Capacity of increasing enzyme activity by *P. chrysosporium*:** Although the catalase content changed minimally in the entire process, urease content increased significantly in the treatment group compared with CK. Increasing urease content could provide a good utilization rate of nitrogen source to plants. Therefore, the inoculation of *P. chrysosporium* into continuous cropping soil would promote plant growth.

**Improvement of the ecological environment of soil.** Given that bacteria always included numerous plant growth-promoting rhizobacteria, whereas pathogenic fungi were more frequently found in sick soil, the quantitative ratio of bacteria to fungi (B/F) is always used to represent soil fertility. The colonization and proliferation of soil-borne fungi may be an important factor in the transition of bacterium-dominated soil to a more fungus-dominated soil (de Vries *et al.*, 2012). After 120 days, the B/F ratios in T20 and T50 were 3.00 and 1.76 times that in CK, respectively, thereby indicating that the inoculation of *P. chrysosporium* could improve the ecological environment of soil for plant growth.

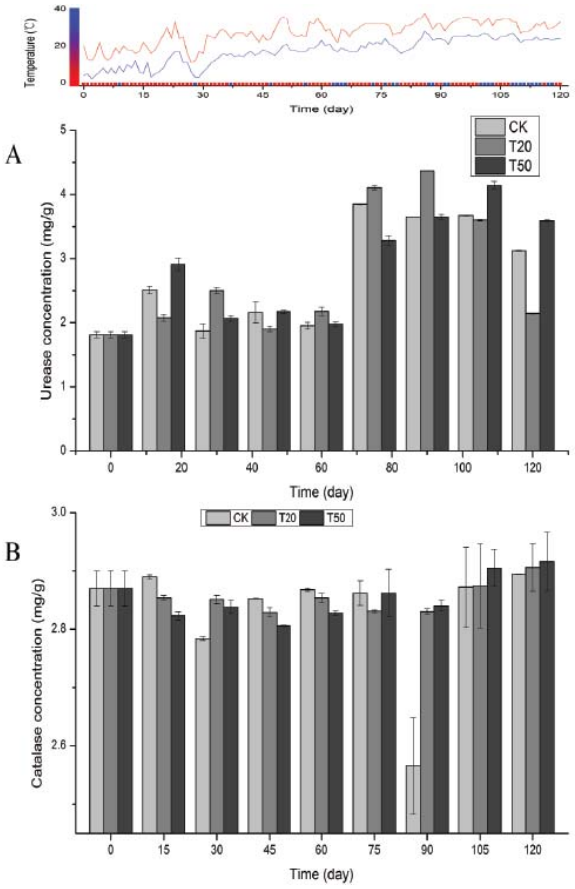


Fig. 3. Change in urease and catalase concentration in the rhizosphere after inoculation of *P. chrysosporium.* CK: control check group; T20, T50: treatment groups.

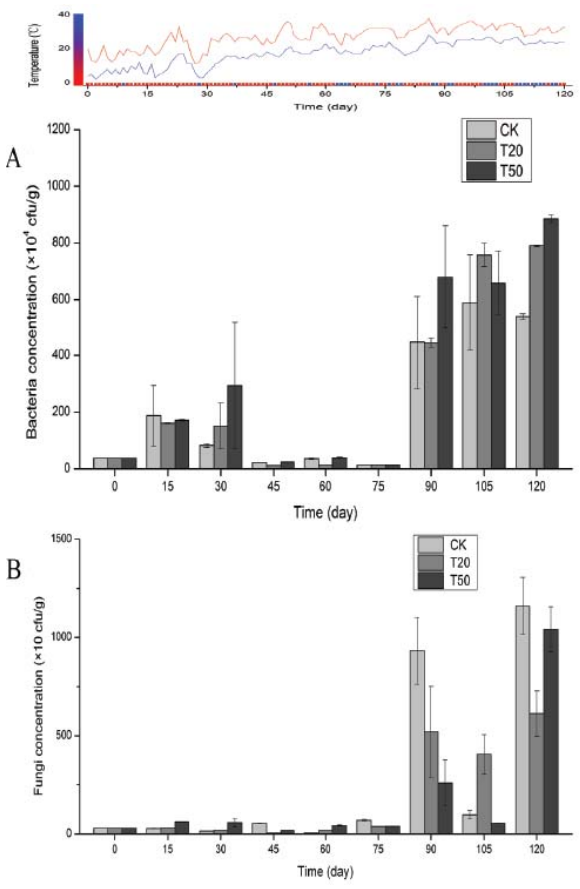


Fig. 4. Change in bacteria and fungi concentration during 120 days of cultivation after inoculation of *P. chrysosporium.* CK: control check group; T20, T50: treatment groups.

**Table 1. Physiological status of *C. morifolium* in CK, T20, and T50 groups after inoculation of *P. chrysosporium*.**

| Time (days) | Group | Leaf length (cm) | Leaf width (cm) | Stem diameter (cm) | Plant height (cm) | Chlorophyll content (cm) |
|---|---|---|---|---|---|---|
| 30 | CK | 5.50 ± 0.94a | 3.47 ± 0.60a | 0.30 ± 0.11ab | 13.49 ± 3.07a | 34.90 ± 4.74ab |
| | T20 | 5.64 ± 0.55a | 3.25 ± 0.47a | 0.32 ± 0.09a | 12.51 ± 2.41ab | 39.67 ± 5.91a |
| | T50 | 4.91 ± 0.66b | 2.85 ± 0.46b | 0.25 ± 0.97b | 11.18 ± 2.09b | 32.31 ± 10.63b |
| 60 | CK | 8.98 ± 0.44a | 5.05 ± 0.63a | 0.53 ± 0.07a | 28.91 ± 2.24b | 42.17 ± 4.23a |
| | T20 | 8.48 ± 0.62a | 4.55a ± 0.72 | 0.54 ± 0.14a | 33.75 ± 4.16a | 45.47 ± 3.40a |
| | T50 | 8.37 ± 0.51a | 4.47 ± 0.76a | 0.59 ± 0.06a | 33.45 ± 2.11a | 43.37 ± 3.59a |
| 90 | CK | 4.16 ± 0.57c | 5.61 ± 0.71a | 0.57 ± 0.12b | 50.08 ± 4.34b | 47.36 ± 3.47a |
| | T20 | 6.55 ±0.38a | 4.53 ± 0.61b | 0.67 ± 0.08a | 59.14 ± 5.39a | 50.33 ± 2.54a |
| | T50 | 4.24 ± 0.84b | 5.61 ± 1.07a | 0.59 ± 0.09b | 51.83 ± 4.98b | 48.88 ± 5.62a |
| 120 | CK | 4.49 ± 0.63a | 3.04 ± 0.36a | 0.58 ± 0.07b | 59.40 ± 4.19b | 53.89 ± 8.64b |
| | T20 | 5.07 ± 0.86a | 3.26 ± 0.58a | 0.68 ± 0.09a | 71.32 ± 1.96a | 62.18 ± 5.70a |
| | T50 | 5.23 ± 0.84a | 3.38 ± 0.50a | 0.65 ± 0.09ab | 60.28 ± 3.33b | 59.01 ± 5.89ab |

Different lower case denotes that the indicators are significantly different >$LSD_{0.05}$

## Conclusion

The quality and productivity of *C. morifolium* is seriously affected during long-term cropping. As a biocontrol method, inoculation of *P. chrysosporium* could effectively degrade autotoxic phenolic acids, such as *p*-hydroxybenzoic acid, vanillic acid, syringic acid, and ferulic acid. This method can also increase the variety of bacterial quantities and inhibit the propagation of fungi, which could ultimately improve the microbial structure and slow down the deterioration of the soil micro-ecosystem. In addition, the approach can increase the urease content, which could promote the transformation of utilizable nitrogen source. The growth condition of continuous cropping *C. morifolium* improved remarkably after inoculation of *P. chrysosporium.* Therefore, *P. chrysosporium* could be a useful tool in alleviating the problem of continuous cropping of *C. morifolium* in the future.

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