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# Dead mycelium of *Penicillium chrysogenum* protects transplanted cotton plants agains fungal wilts in a saline field

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## Abstract

Previous studies have shown that the dead mycelium of *Penicillium chrysogenum* at 900-1,500 kg ha$^{-1}$ effectively controlled Fusarium and Verticillium wilt of cotton. Our objective was to determine if the dead mycelium is also effective at lower rates under a cotton seedling transplanting system. Columned soil blocks (500 g each), made of fertile soil alone in the first experiment, and both soil and dead mycelium at varying rates in the second experiment, were sown with cotton seeds in 2006 and 2007. Seedlings in the mycelium-free blocks were drenched with aqueous extract of the dead mycelium after full emergence, and then transplanted to a saline field. The disease severity of *Fusarium oxysporum* f.sp *vasinfectum* and *Verticillium dahliae*, leaf chlorophyll and malondialdehyde concentrations, leaf photosynthetic rate and lint yield of the transplanted plants were determined. The aqueous extract at a rate of 1 to 5% provided 18.6 to 25.6% protection against Fusarium wilt but not against Verticillium wilt. Lint yield was slightly increased with the aqueous extract in 2006, but not in 2007. The incorporation of the dead mycelium to soil blocks before sowing was effective in controlling both the Fusarium and Verticillium wilts of cotton. Dead mycelium applied at 1 to 3% (w/w) provided 26-30% protection against Fusarium wilt and 48-50% protection against Verticillium wilt, and increased lint yield by 13-14%. Such incorporation also delayed leaf senescence as indicated by the increased leaf photosynthetic rate and chlorophyll content, and reduced malondialdehyde concentrations. Incorporation of the dead mycelium of *P. chrysogenum* into soil blocks at a relatively lower rate (270 kg ha$^{-1}$) would be an effective application mode for wilt control in sustainable cotton production.

**Additional key words**: *Fusarium oxysporum* f.sp *vasinfectum*; leaf senescence; lint yield; seedling transplanting; *Verticillium dahliae.*

## Resumen

### Micelio muerto de *Penicillium chrysogenum* protege contra marchitamientos fúngicos en plantas de algodón trasplantadas a un campo salino

Estudios anteriores han demostrado que el micelio muerto de *Penicillium chrysogenum* a 900-1.500 kg ha$^{-1}$ controla eficazmente los marchitamientos de algodón producidos por Fusarium y Verticillium. Nuestro objetivo fue determinar si el micelio muerto es también eficaz a tasas más bajas utilizando plántulas de algodón trasplantadas. Bloques de columnas de suelo de 500 g, con solo tierra fértil en un primer experimento, y suelo+micelio muerto en diferentes cantidades en un segundo experimento, se sembraron con semillas de algodón en 2006 y 2007. Tras la emergencia, las plántulas de los bloques sin micelio fueron empapadas con extractos acuosos de micelio muerto y se trasplantaron a un campo salino. Se determinó la gravedad de la enfermedad de *Fusarium oxysporum* f.sp *vasinfectum* y *Verticillium dahliae*, la clorofila de las hojas, la concentración de malondialdehído, la tasa de fotosíntesis foliar y el rendimiento de hilas de las plantas trasplantadas. Un 1-5% de extracto proporcionó una protección del 18,6-25,6% contra Fusarium, pero no contra la verticilosis. El rendimiento de hilas se incrementó ligeramente con el extracto acuoso en 2006, pero no en 2007. La incorporación de micelio muerto a los bloques de suelo antes de sembrar fue eficaz en el control tanto de Fusarium como de Verticillium. Micelio muerto aplicado al 1-3% (p/p), proporcionó una protección del 26-30% contra Fusarium y del 48-50% contra Verticillium, y aumentó la producción de hilas un 13-14%. Esta incorporación también retrasó la senescencia foliar, como lo indica un aumento de la tasa de fotosíntesis foliar

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y un mayor contenido de clorofila, así como una reducción de la concentración de malondialdehído. La incorporación de micelio muerto de *P. chrysogenum* en bloques de suelo a una tasa relativamente baja (270 kg ha$^{-1}$) puede servir para el control de la marchitez en la producción de algodón sostenible.

**Palabras clave adicionales**: *Fusarium oxysporum* f.sp *vasinfectum;* senescencia de las hojas; rendimiento de hilas de algodón; trasplante de plántulas; *Verticillium dahliae.*

## Introduction

Cotton (*Gossypium hirsutum* L.) is a global cash crop. It is attacked by a wide range of economically important fungal pathogens. Fusarium and Verticillium wilts caused by *Fusarium oxysporum* f.sp *vasinfectum* and *Verticillium dahliae* Kleb, respectively, are the most destructive diseases for cotton in China (Davis *et al*., 1996; Jian *et al*., 2003). The diseases are usually managed by applying fungicides like Carbendazim, using cotton cultivars with improved disease resistance or tolerance, or farming practices like rotation (Jian *et al*., 2003). However, since none of the current upland cotton cultivars are completely resistant to *V. dahliae* (Colson-Hanks *et al*., 2000), and both the pathogens can remain viable in soil for several years, they are difficult to control by using current commercial cultivars or cultural practices alone. Applying synthetic fungicides may cause soil and environment pollution in cotton fields. Therefore, it is necessary to develop new methods or new products to help in controlling the diseases.

The use of environmentally friendly biological products is a new and promising approach for controlling cotton diseases (Brannen and Kenney, 1997). Dead mycelium of *Penicillium chrysogenum* is a fungal biomass obtained from industry after extraction of penicillin. The dead mycelium (powder) from *P. chrysogenum* and its aqueous extract effectively controlled *F. oxysporum* f.sp *vasinfectum* and *V. dahliae* in potted cotton plants under glass-house conditions (Dong and Cohen, 2002a,b; Dong *et al*., 2003). In field miniplots inoculated with *F. oxysporum* f.sp *vasinfectum* or *V. dahliae,* Saidakarimov and Cohen (2003) found that the dead mycelium incorporated into soil protected cotton against their respective wilts. Gotlieb *et al*. (2003) reported that the dead mycelium protected cucumber and tomato plants against the root-knot nematode, *Meloidogyne javanica* in a shaded house. Foliar spray of aqueous extract of the dead mycelium protected grapevine from downy and powdery mildew, tomato from early blight, onion from downy mildew and apple trees from apple scab in controlled and field conditions (Thuerig *et al*., 2006). Our more recent study showed that pre-planting application plus top dressing with the dead mycelium powder at rates of 900 to 1,500 kg ha$^{-1}$ provided protection of 35-43% against *F. oxysporum* f.sp *vasinfectum*, and of 48-50% against *V. dahliae*, and increased lint yield by ~9% (Dong *et al*., 2006). However, the product has not been widely used for disease control in cotton production mainly due to the high rate of application and the associated cost.

Transplanting is a widely used cultural practice for cotton production in China (El-Sahrigi *et al*., 2001; Dong *et al*., 2005, 2007). In this system, cotton seeds are sown in columned soil blocks and allowed to grow in a greenhouse-like hut during the early season, and then transplanted to open fields after the temperature rises (Dong *et al*., 2005). The transplanting method significantly enhanced yield, reduced seeding rate and improved stand establishment by providing beneficial environment before transplanting, and extending the duration of growth and development in comparison with the conventional planting of cotton in low temperature areas (Dong *et al*., 2005). Such advantages of cotton transplanting have also been reported in other countries (Sherif *et al*., 1995; Karve, 2003).

Dead mycelium of *P. chrysogenum* is a promising fungal product for disease control, but the high rate of application and the associated cost has limited its wide application in the fields. Thus it is very important to search new application modes at a reduced rate and cost for the product. One objective of this study was to determine if the application of dead mycelium of *P. chrysogenum* at a low rate to soil blocks before sowing could effectively control wilt disease and increase lint yield in a seedling transplanting system; the other objective was to determine if the yield increase due to the dead mycelium was mainly attributed to the disease-control effect through delayed leaf senescence.

Abbreviations used: Chl (chlorophyll); Ck (control); DM (dead mycelium); ECe (electrical conductivity of soil saturation extract); MDA (malondialdehyde); Pn (photosynthetic); RBD (random block design).

## Material and methods

### Preparation of powder and extract

Dead mycelium of *P. chrysogenum* was obtained from Biochemie Ltd., Kundl, Austria. The fungal biomass was dried by the manufacturer for 4 h at 110°C and contained no living mycelium (Dong *et al*., 2006). The mycelium powder was further autoclaved to kill possible living organisms before use in the present experiment. The mycelium powder contained 7% N, 1% P and 2% K.

An aqueous extract of the dead mycelium was prepared using the following procedure: 100 g of mycelial powder was suspended in 1,000 mL sterile distilled water. The suspension was shaken for 2 h at 100 rpm and then stored for 22 h at room temperature. It was then briefly agitated and filtered through Whatman No. 1 filter paper (Sanger Biotech, China). After cooling, the dead mycelium filtrate (10%) was stored as stock solution at 4°C.

### Experimental site and cotton cultivar

The experiment was conducted in a slightly saline cotton field in Huimin County (117° 51' E, 37° 49' N) in 2006 and 2007. The field had a sandy loam soil with an ECe 7.12 dS $m^{-1}$ and contained 10.8 g $kg^{-1}$ organic matter, 47.9 mg $kg^{-1}$ available N, 14.5 mg $kg^{-1}$ available P and 156 mg $kg^{-1}$ available K. The field has been successively planted with cotton for 30 years with a history of heavy incidence of Fusarium and Verticillium wilts of cotton. LU643, a cotton cultivar susceptible to both Fusarium wilts, was used in this study.

### Raising of seedlings and treatment

Cotton seedlings were raised in a nursery bed and then transplanted into fields for further growth and development. For raising seedlings, "columned soil blocks" (500 g in weight and 4 cm in diameter each), made of non-saline fertile soil (ECe ≤ 2.8 dS $m^{-1}$), were prepared by hand with a mold in early April before planting. Soil blocks were then tidily placed into a eutropic soil bed (10 cm deep and 2 m wide). After watering, each block was sown with 3 cotton seeds. The seedling bed was then covered with plastic film (12 μm in thickness) which was supported by bamboo sticks to build a 50 cm-high arciform hut. Seedling plants with the second true leaves expanded were transplanted.

The mycelial powder treatments were administered by mixing different quantities of the powder product with soil during molding of columned blocks. Columned soil blocks containing 0.5, 1.0, 2.0 and 3.0% (w/w) served as four powder treatments, while those without the dead mycelium served as the control. In order to avoid possible interference of main mineral nutrient from the dead mycelium, soils for the control (Ck), 0.5, 1.0 and 2.0% dead mycelium treatments were adjusted with urea, superphosphate and $K_2SO_4$ to have equal levels of N, P and K to that for 3.0% mycelium treatment before molding.

Aqueous extract treatments were conducted by drenching blocks with different concentrations of the extract using a pipette. Extracts of 1.0, 2.0, 3.0 and 5% were drenched into blocks (30 mL per block) after thinning. Soil drench with an equal volume of distilled water was used as the controls.

### Seedling transplanting and experimental design

When most seedlings reached the two-true leaf stage, soil blocks along with seedlings were transplanted to the field plots by hand with a transplanter. The experiment was arranged into a random block design (RBD) with four replications. Each plot contained 6 rows of cotton, 15 m long; the row spacing was 0.80 m. For both the powder and extract experiments, the plant density in each field plot was 4.5 plants $m^{-2}$, which is the typical plant density for cotton production in the locality. Soon after transplanting, each field plot was watered to let seedlings recover normal growth. Cotton fields were managed according to local agronomic practices unless otherwise indicated.

### Physiological assays

Ten consecutive plants in an inner row per plot in both control and 1% mycelial powder treatments were selected at the beginning of boll opening. Net photosynthetic rates, concentration of chlorophyll and lipid peroxidation of the second leaf on main-stem from terminal were determined as an indication of leaf senescence. The photosynthetic rate was measured between

09:00 and 11:00 on cloudless days when ambient photosynthetic photon flux density exceeded 1,500 mmol $m^{-2}$ $s^{-1}$), using an LI-6400 portable photosynthesis system (Li-Cor, Lincoln, NE, USA). Leaf chlorophyll was extracted over a 24 h period in darkness at 30°C with ethanol and acetone, and measured with a spectrophotometer (TU1901, Beijing Purking General Instrument Co. Ltd.) at 665 and 750 nm. The peroxidation of leaf lipids was measured from the malondialdehyde content following procedures described in Iturbe-Ormaetxe *et al*. (1998).

### Assessment of disease severity and yield

According to previous investigation (Jian *et al*., 2003), both pathogens of *F. oxysporum* f.sp *vasinfectum* and *V. dahliae* usually attack cotton plants from June to July and after late July, respectively. Twenty plants per plot were randomly selected and removed from the soil at peak boll-setting (early August) for Fusarium and at harvest for Verticillium assessment. The plants were delivered to the National Diagnosis Center for Plant Diseases, Jinan, Shandong, where plants infected by either *F. oxysporum* f.sp *vasinfectum* or *V. dahliae* were identified. Disease severity of Fusarium or Verticillium wilt was assessed using a stem cut rating system (Dong *et al*., 2006). Briefly, each plant was scored for stem discoloration using a 0-4 scale: 0 = no discoloration; 1 = 1/4 of the cross section showing discoloration; 2 = 1/2 of the cross section showing discoloration; 3 = 3/4 of the cross section showing discoloration; 4 = full cross section showing discoloration. Disease severity was expressed as a disease severity index = Σ (the number of plants with a disease score × the value for the corresponding score)/(total number of plants rated × the highest scale value). Percentage protection was then calculated as (1-*a/b*) when *a* is % disease in treated plants and *b* is % disease in control plants.

Seed cotton was harvested four times by hand picking, and lint yield was determined after ginning.

### Statistical analysis

Analysis of variables was performed with the function of RBD analysis using DPS Data Processing System (Tang and Feng, 1997). If neither the year factor nor the interaction between year and the dead mycelium was significant, the two years' data were combined for analysis. However, when year factor was significant, only one year data was chosen as representative of the two years. Mean separations were completed using Tukey's test at a significance level of 0.05 or 0.01. Figures were drawn using Excel 2003 (Microsoft, Bothell, WA, USA).

## Results

### Effect of aqueous extract of dead mycelium on wilt severity and yield

Field monitoring showed that diseased plants by *F. oxysporum* f.sp *vasinfectum* were stunted, followed by yellowing of the leaves and loss of foliage. These plants fruited earlier than normal with smaller bolls that opened prematurely. Soil drenching with the aqueous extract of dead mycelium into the seedling bed provided significant protection for the transplanted plants against Fusarium wilt in both years, based on disease index and percentage protection (Table 1). Although the controlling efficacy to Fusarium wilt was affected by mycelium concentration and experimental year, no significant interaction between both factors was observed. Soil drenching with the extract ranging from 1 to 5% provided 19 to 26% protection against Fusarium wilt in 2006. Similar percentage protection was obtained in 2007 (Table 1). Based on efficacy and cost, a concentration of 3% seemed to be better for control of Fusarium wilt.

Field monitoring also showed that symptoms of Verticillium wilt initially occurred on the lower leaves at peak flowering and became severe at peak boll-setting. Affected plants were stunted and leaves turned yellow at the margin and areas between the main veins. These areas eventually died, leaving leaves with a scorched appearance. Soil drenching with the aqueous extract ranging from 1 to 5% did not significantly protect the transplanted plants against Verticillium wilt either year (Table 1). The results suggest that aqueous extract of the dead mycelium applied at the seedling stage would not significantly control Verticillium wilt under field conditions.

A slight increase (3.7-4.4%) in lint yield was found in treated plants with the aqueous extract relative to the non-treated control plants in 2006, but no significant increase was obtained with aqueous extract ranging from 1 to 5% in 2007 (Table 1).

**Table 1.** Effect of aqueous extract of dead mycelium (DM) of *Penicillium chrysogenum* on wilt incidence and lint yield of cotton

| Aqueous extract of mycelium | Fusarium wilt | | Verticillium wilt | | Yield | |
|---|---|---|---|---|---|---|
| | Disease index | Protection (%) | Disease index | Protection (%) | Lint yield (kg ha$^{-1}$) | Increase (%) |
| *2006* | | | | | | |
| Ck | 16.8$^{a}$ | — | 21.5$^{a}$ | — | 1,277$^{b}$ | — |
| 1% | 13.6$^{b}$ | 19.0 | 21.3$^{a}$ | 0.9 | 1,324$^{ab}$ | 3.7 |
| 2% | 13.0$^{bc}$ | 22.6 | 21.3$^{a}$ | 0.3 | 1,350$^{a}$ | 5.7 |
| 3% | 12.4$^{c}$ | 26.2 | 21.0$^{a}$ | 2.3 | 1,345$^{a}$ | 5.3 |
| 5% | 12.9$^{c}$ | 23.2 | 21.4$^{a}$ | 0.5 | 1,333$^{a}$ | 4.4 |
| *2007* | | | | | | |
| Ck | 17.5$^{a}$ | — | 20.9$^{a}$ | — | 1,249$^{a}$ | — |
| 1% | 14.3$^{b}$ | 18.3 | 20.6$^{a}$ | 1.4 | 1,257$^{a}$ | 0.6 |
| 2% | 13.7$^{bc}$ | 21.7 | 20.4$^{a}$ | 2.4 | 1,264$^{a}$ | 1.2 |
| 3% | 13.2$^{c}$ | 24.6 | 20.3$^{a}$ | 2.9 | 1,265$^{a}$ | 1.3 |
| 5% | 13.4$^{c}$ | 23.4 | 20.5$^{a}$ | 1.9 | 1,273$^{a}$ | 1.9 |
| *Combined* | | | | | | |
| Ck | 17.2$^{a}$ | — | 21.2$^{a}$ | — | 1,263$^{b}$ | — |
| 1% | 14.0$^{b}$ | 18.6 | 21.0$^{a}$ | 0.9 | 1,290$^{ab}$ | 2.1 |
| 2% | 13.4$^{c}$ | 22.1 | 20.9$^{a}$ | 1.4 | 1,307$^{a}$ | 3.5 |
| 3% | 12.8$^{d}$ | 25.6 | 20.7$^{a}$ | 2.4 | 1,305$^{a}$ | 3.3 |
| 5% | 13.2$^{cd}$ | 23.3 | 21.0$^{a}$ | 0.9 | 1,303$^{a}$ | 3.2 |
| *Source of variance* | | | | | | |
| Year (Y) | 0.0001 | | 0.0024 | | 0.3182 | |
| DM | $<0.0001$ | | 0.5427 | | 0.0032 | |
| Y × DM | 0.9692 | | 0.9772 | | 0.1864 | |

Aqueous extract of the DM was applied with soil drench in the seedling bed after thinning before transplanting. Treatment with water served as the control (Ck). For each year, means within the same column followed by different letters differ significantly according to Duncan's multiple range test ($p < 0.05$).

## Effect of dead mycelium powder on wilt severity and yield

The mycelium powder incorporated into soil blocks significantly inhibited Fusarium wilt in both 2006 and 2007 (Table 2). Although the control efficacy was greatly affected by dose of application and the experimental year, there was no significant dose × year interaction effect on control efficacy. Application rates ranging from 0.5 to 3.0% provided 24-33% protection against Fusarium wilt in 2006. The rate of 1% seemed to be better for disease control and cost-saving.

Unlike aqueous extract, the powder incorporated into soil blocks also significantly controlled Verticillium wilt in both years (Table 2). The control efficacy varied with application rate and experimental year, but their interaction was not significant. Application rates ranging from 0.5 to 3.0% provided 27-48% protection against Verticillium wilt in 2006. The rate of 1% appeared to be better than others for disease control and cost-saving.

Increase of lint yield was obtained by the mycelium powder in both years (Table 2); the powder rate of 0.5% significantly increased lint yield by 8.2%, while other treatments resulted in more yield increase (~13%). A dosage of 1% also appeared to be better for increasing yield.

## Effect of dead mycelium powder on leaf senescence

Physiological analysis of the 2$^{nd}$ main-stem leaf at the start of boll-opening from terminal showed that the

**Table 2.** Effect of dead mycelium powder of *Penicillium chrysogenum* on wilt incidence and lint yield of cotton.

| Dead mycelium powder | Fusarium wilt | | Verticillium wilt | | Yield | |
|---|---|---|---|---|---|---|
| | Disease index | Protection (%) | Disease index | Protection (%) | Lint yield (kg ha$^{-1}$) | Increase (%) |
| *2006* | | | | | | |
| Ck | 17.7$^{a1}$ | — | 24.1$^{a}$ | — | 1,245$^{c}$ | — |
| 0.5% | 13.4$^{b}$ | 24.3 | 17.7$^{b}$ | 26.6 | 1,315$^{b}$ | 5.6 |
| 1.0% | 12.1$^{c}$ | 31.6 | 12.9$^{c}$ | 46.5 | 1,390$^{a}$ | 11.6 |
| 2.0% | 11.9$^{c}$ | 32.8 | 12.7$^{c}$ | 47.3 | 1,388$^{a}$ | 11.5 |
| 3.0% | 12.2$^{c}$ | 31.1 | 12.6$^{c}$ | 47.7 | 1,392$^{a}$ | 11.8 |
| *2007* | | | | | | |
| Ck | 15.6$^{a}$ | — | 23.2$^{a}$ | — | 1,108$^{c}$ | — |
| 0.5% | 12.2$^{b}$ | 21.8 | 15.3$^{b}$ | 34.1 | 1,232$^{bc}$ | 11.2 |
| 1.0% | 11.4$^{b}$ | 26.9 | 11.9$^{c}$ | 48.7 | 1,283$^{ab}$ | 15.8 |
| 2.0% | 11.2$^{b}$ | 28.2 | 11.5$^{c}$ | 50.4 | 1,291$^{a}$ | 16.5 |
| 3.0% | 12.4$^{b}$ | 20.5 | 11.1$^{c}$ | 52.2 | 1,278$^{ab}$ | 15.3 |
| *Combined* | | | | | | |
| Ck | 16.7$^{a}$ | — | 23.7$^{a}$ | — | 1,177$^{c}$ | — |
| 0.5% | 12.8$^{b}$ | 23.4 | 16.5$^{b}$ | 30.4 | 1,274$^{b}$ | 8.2 |
| 1.0% | 11.8$^{c}$ | 29.3 | 12.3$^{c}$ | 48.1 | 1,336$^{a}$ | 13.5 |
| 2.0% | 11.6$^{c}$ | 30.5 | 12.2$^{c}$ | 48.5 | 1,340$^{a}$ | 13.8 |
| 3.0% | 12.3$^{bc}$ | 26.3 | 11.9$^{c}$ | 49.8 | 1,335$^{a}$ | 13.4 |
| *Source of variance* | | | | | | |
| Year (Y) | 0.0013 | | 0.0010 | | 0.0004 | |
| DM | < 0.0001 | | < 0.0001 | | 0.0021 | |
| Y × DM | 0.0762 | | 0.6529 | | 0.7118 | |

[1] Powder of DM was incorporated into soil before blocks making. Soil blocks containing no DM served as the control. For each year, means within the same column followed by different letters differ significantly according to Duncan's multiple range test ($p < 0.05$).

powder (1%) incorporated into soil blocks significantly increased chlorophyll, by 40 and 34%, and decreased malondialdehyde by 13.9 and 17.1% in 2006 and 2007, respectively (Fig. 1). The powder also improved photosynthetic rate of the main-stem leaf by 38.5 and 30.7%. Leaf senescence seems to be greatly delayed by the dead mycelium powder in terms of improved photosynthesis and reduced lipid peroxidation.

## Discussion

Earlier studies conducted under greenhouse or shade-house conditions showed that an application of the dead mycelium of *P. chrysogenum* or its aqueous extract could protect corn from *Fusarium moniliforme* (Gao *et al*., 2001), melon from *F. oxysporum* f.sp. *melonis* (Dong and Cohen, 2001), cotton from Fusarium and Verticillium wilts (Dong and Cohen, 2002a; Saidkarimov and Cohen, 2003) and tomato or cucumber from the root-knot nematode *M. javanica* (Gotlieb *et al*., 2003). In the present experiments, both aqueous extract and powder of the dead mycelium were tested for their control effects on Fusarium and Verticillium wilts of cotton under saline field conditions. Aqueous extract from 1 to 5% applied in the nursery bed provided 18.6 to 25.6% protection against Fusarium wilt, but did not show any significant control of Verticillium wilt. Lint yield was slightly increased (4.4-5.7%) with ≥ 2% aqueous extract of mycelium in 2006, but not in 2007. However, the effects of the dead mycelium powder on wilt disease control and yield increase were much more promising than its aqueous extract. The powder applied to soil blocks before sowing was effective in

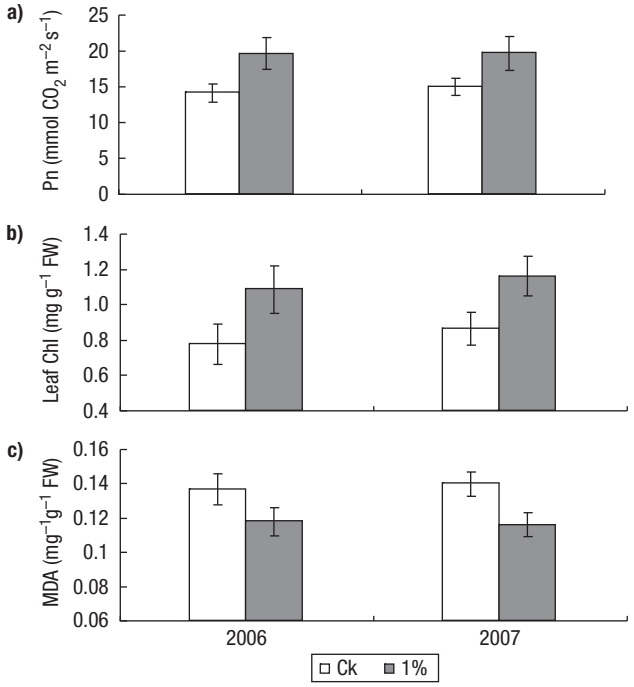


**Figure 1.** Effect of dead mycelium powder of *Penicillium chrysogenum* on photosynthetic rate (Pn) (a) and chlorophyll (Chl) (b) and malondialdehyde (MDA) contents (c) in cotton leaves. Error bars show ± SD.

controlling both the Fusarium and Verticillium wilts of transplanted plants under field conditions. Averaged across the two years of study, the mycelium powder from 1 to 3% provided 26-30 and 48-50% protection against Fusarium and Verticillium wilts, respectively. The powder (1-3%) also increased lint yield by 13-14%. Since control efficacy against the two pathogens and the yield increment with the dead mycelium were not significantly different between the 1% rate and the other two higher rates, the 1% rate would be the optimal use rate for mycelium powder against wilt in the transplanting production system.

A previous study (Dong *et al*., 2006) showed that pre-planting application plus top dressing with the mycelium powder at 90 and 150 g $m^{-2}$ (900 and 1,500 kg $ha^{-1}$) provided protection of 34.6 and 42.7% against Fusarium wilt, and 47.8 and 49.6% against Verticillium wilt, and increased lint yield by 9 and 9.2%. The dead mycelium powder incorporated into soil blocks at the rate of 1% in the present study exhibited a similar disease-control efficacy, and better yield increment than obtained from our previous study (Dong *et al*., 2006). The typical plant density for the experimental area was about 45,000 plants $ha^{-1}$. A total of ~54,000 soil blocks were consumed per hectare due to failure of seed emergence, and seedling disease in nursery bed. Thus, the mycelium powder applied at the rate of 1% per hectare (54,000 soil blocks) under the seedling transplanting system was 270 kg, being 2.3 and 4.6 times lower than rates used for soil amendment in our previous study (Dong *et al*., 2006). Total costs of supplying and applying the mycelium powder were greatly reduced. Thus, the powder incorporated into soil blocks is an efficient application mode for disease control and yield improvement due to its high disease-control efficacy at low cost.

Many disease control measures including use of fungicides or biological agents could enhance plant growth and yields (Hanson, 2000), whereas some others, like the use of the resistance inducer INA (2,6-dichloroisonicotinic acid) or BTH (benzothiadiazole), were reported to have phytotoxic properties on crops (Sticher *et al*., 1997). Such responses of growth or yield enhancement were usually attributed to an indirect effect associated with control of plant diseases. The use of dead mycelium of *P. chrysogenum* containing 7% N, 1% P and 2% K as disease control agent enhanced the growth of sweet corn and cotton under controlled conditions (Gao *et al*., 2001; Dong and Cohen, 2002b). In the present study, since all the treatments including the mycelium-free controls were adjusted to an equal level of the major nutrients (N, P and K) with inorganic fertilizers, the yield gains by the dead mycelium were mainly derived from its disease-control effect. Nevertheless, the possible role of organic matter and trace elements in the dead mycelium can not be completely excluded.

*Fusarium oxysporum* f.sp. *vasinfectum* and *Verticillium dahliae* pathogens penetrate the cotton root and systemically infect the plant through the xylem (Daayf *et al*., 1997; Hanson, 2000). Infected plants usually exhibit symptoms of marginal chlorosis or necrosis in leaves, discoloration of the stem vascular bundles, decrease in photosynthesis and increase in respiration, thus resulting in a significant reduction in plant biomass and heavy loss of yield (Hampton *et al*., 1990). In the present study, dead mycelium powder (1%) significantly increased concentration of chlorophyll and net photosynthetic rate, and reduced malondialdehyde content in the main-stem leaves. Because of the improved photosynthesis and reduced lipid peroxidation, leaf senescence seemed to be greatly delayed by the dead mycelium powder through inhibition of the Verticillium and Fusarium wilt diseases. The results further confirmed that the yield increase was mainly due

to the disease-control effect of dead mycelium of *P. chrysogenum*.

Our previous study *in vitro* (Dong *et al*., 2006) showed no inhibitory effect of dead mycelium of *P. chrysogenum* on mycelial growth of *V. dahliae* and *F. oxysporum* f.sp. *vasinfectum*. Therefore, it is suggested that the protection of cotton plants against these two pathogens was probably attributable to induced resistance by dead mycelium of *P. chrysogenum*. Further evidence for induced resistance was that dead mycelium of *P. chrysogenum* induced the accumulation of pathogenesis-related (PR) protein transcripts and enhanced peroxidase activity in cotton plants (Dong and Cohen, 2002a,b; Dong *et al*., 2006, 2007). Peroxidase is known to be involved in induced resistance due to its role in lignification of plant cell walls (Dong *et al*., 2003). However, Gao *et al*. (2001) found that dead mycelium of *P. chrysogenum* inhibited propagation of *F. moniliforme* in soil, and Gotlieb *et al*. (2003) reported that protection against root-knot nematode in cucumber and tomato did not operate via induced resistance. This was because the dead mycelium immobilized nematode juveniles and reduced the egg hatching rate in vitro. Therefore, a possible anti-fungal effect under field conditions should not be completely excluded.

As conclusions. the dead mycelium of *P. chrysogenum* incorporated into soil blocks at a dosage of 270 kg $ha^{-1}$ effectively controlled Fusarium and Verticilium wilt diseases and significantly increased lint yield (~13%) of transplanted cotton. The yield increase was mainly attributed to the delayed leaf senescence as a result of disease control by dead mycelium of *P. chrysogenum*. The biological product can be a promising tool for control of Verticillium and Fusarium wilt diseases in sustainable cotton production.

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