Published in final edited form as:
. 2012 September ; 134(1): 87–95. doi:10.1007/s10658-012-0024-3.

Control of Fusarium wilt in banana with Chinese leek

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Abstract

The inhibitory effects of Chinese leek(*Allium tuberosum*) on *Fusarium oxysporum* f. sp. *cubense* (*Foc*) and on *Fusarium* wilt incidence were studied in order to identify a potential efficient way to control the disease. Adopting the rotation system of Chinese leek-banana reduced the Fusarium wilt incidence and disease severity index by 88 %-97 % and 91 %-96 %, respectively, improved the crop value by 36 %-86 %, in an area heavily infested by *Foc* between 2007 and 2009. As a result of inoculation in the greenhouse, Chinese leek treatment reduced disease incidence and the disease severity index by 58 % and 62 %, respectively in the variety Baxi (AAA) and by 79 % and 81 %, respectively in the variety Guangfen NO.1 (ABB). Crude extracts of Chinese leek completely inhibited the growth of *Foc* race 4 on Petri dishes, suppressed the proliferation of the spores by 91 % and caused 87 % spore mortality. The findings of this study suggest that Chinese leek has the potential to inhibit *Foc* growth and *Fusarium* wilt incidence. This potential may be developed into an environmentally friendly treatment to control *Fusarium* wilt of banana.

Keywords

Fusarium oxysporum; Panama disease; banana; *Allium tuberosum*; crop rotation; Biocontrol

Introduction

Fusarium wilt of banana (also called Panama disease), is caused by the soil-borne hyphomycete, *Fusarium oxysporum* f. sp. *cubense* (*Foc*). It is one of the most notorious banana diseases (Ploetz 2000;Stove 1962). *Foc* race 4 has seriously affected commercial plantations of Cavendish in Taiwan, Northern Territory of Australia, Indonesia, Malaysia, Canary and Madeira Islands, South Africa and Chinese mainland, making their banana export less competitive (Molina et al. 2008; Ploetz, 2006). Mainly due to this disease, the banana production area in Taiwan declined from 50,000 ha in the 1960s to 5,000 ha in the early 1970s (Hwang and Ko 2004). The disease destroyed the banana industries in the main producing areas in Chinese mainland, including Guangdong, Guangxi, Hainan, Fujian and Yunnan (Lian et al. 2009). Currently, the disease incidence in the delta of the Pearl River (a major banana production area in China), ranges between 20 to 40 %, with individual plantations reaching a rate of 90%.

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Scientists around the world have investigated resistance breeding (Hwang and Ko 2004 ; Smith et al.2006), chemical control (Nel et al. 2007), biocontrol (Saravanan et al. 2003; Cao et al.2005; Lian et al.2009) and molecular methods (Paul et al. 2011; Yip et al. 2011),in search of methods to control the disease. Some of these studies were found to effectively suppress *Foc* growth in the laboratory and in greenhouses, but were unable to efficiently control *Fusarium* wilt in the field.

In China, in Panyu, Guangzhou, an area heavily infested by *Foc* bananas are rotated with 2-3 years of commercially cultivated Chinese leek (*Allium tuberosum*)to control *Fusarium* wilt . This rotation was first identified by chance in 1997 by a farmer whose bananas were heavily infected by *Foc* with the exception of a section of the plantation where Chinese leeks had been grown for a few years previously. Several farmers have tried this system with great success since.

The objectives of this study were (1): to verify if *Fusarium* wilt incidence in banana can be decreased by rotation with Chinese leek in the field, and (2) to provide a quantitative estimate of the potential of this method for the control of the *Fusarium* wilt based on greenhouse and laboratory studies.

Materials and Methods

Site

Panyu area (22°45′-23°05′ N, 113°14′-113°34′) is located in the south of Guangzhou, China. It is characterized by a subtropical marine monsoon climate with a mean annual precipitation of about 1900 mm and a mean annual temperature of 21.6°C. This area, once one of the main production areas of bananas in China, now is heavily infested by *Foc,* and many banana orchards have been planted with other crops due to the high incidence of *Fusarium* wilt.

Plant materials and Fungi

The banana cultivars used in the experiment were banana variety Baxi (*Musa* AAA Cavendish subgroup) and Guangfen No.1 (*Musa* ABB group) which are highly susceptible to *Foc*. They were provided by The Banana Tissue Culture Center, Institute of Fruit Tree Research, Guangdong Academy of Agriculture Science. Cupped plantlets of 30mm height were used in the greenhouse and field tests. Fresh Chinese leek (*Allium tuberosum* cv Xuejiu) was collected from commercial neighbouring fields. *Foc* race4 isolate CGMCCC 3.12196 was provided by the Key Laboratory of South Subtropical Fruit Biology and Genetic Resource Utilization, Ministry of Agriculture, China.

Field studies

The field trials were carried out with the cooperation of local farmers in Panyu area (Dagang town, Yuwotou town, Dongchong town) between 2007 and 2009. Fields where Chinese leek had just been continuously grown for 3 years were selected as the Chinese leek treatment to evaluate the inhibitory effect of Chinese leek on the incidence of *Fusarium* wilt. More than 4.5 ha of treated fields were involved in the trial in 2007, 12.8 ha in 2008 and 21.5 ha in 2009, respectively. Neighbouring fields where other vegetables (3 ha in 2007), sugarcane (23 ha in 2008) and rice (1 ha in 2009) had been grown were selected as control fields. All these selected fields were heavily infested by *Foc* and could not be used to plant banana because of high incidence of Fusarium wilt before cultivating Chinese leek or other crops. In late March and early April each year, after removing the Chinese leek and other crops, three month old 30 mm high Guangfen No.1 (ABB) plantlets were transplanted in the treated and the control fields with a density of 2.0 m × 2.7 m. All plants were managed in the same way and no artificial inoculation was performed. At harvest, the number of infected plants in each plot was recorded each year to evaluate the disease incidence. Per plot, 100 plants were randomly selected and the disease symptoms rated on each plant based on five classes from 0 to 4 modified after Mak (Mak et al.2004): 0 - No streaking or yellowing of leaves, plant appears healthy; 1 - Slight streaking and/or yellowing of lower leaves; 2 - Streaking and/or yellowing of most of the lower leaves; 3 - Extensive streaking and/or yellowing on most or all of the leaves; 4 - Dead plant. Based on the infection counts and the disease symptom assessments the disease incidence and a disease severity index were calculated as follows: Disease incidence (%) =(Number of infected plantlets / Total number of plantlets)×100. Disease severity index =[∑ (Class × Number of plants in that class) / (4 × Total number of assessed plants)] ×100. In addition, yield and crop value were assessed. All trials were replicated three times.

Greenhouse trial

A green house trial was conducted with two banana varieties with and without Chinese leek in peat soil inoculated with *Foc* , i.e. four treatments: (1) Baxi treatment: Baxi (AAA) +Chinese leek+ *Foc*; (2)Baxi control: Baxi (AAA) + *Foc*;(3) Guangfen No.1 treatment: Guangfen No.1 (ABB) +Chinese leek+ *Foc*;(4)Guangfen No.1 control: Guangfen No.1 (ABB) + *Foc*.

About 2.0 kg of aerial parts of fresh Chinese leek were cut into 2-5 cm long pieces, and mixed evenly with about 10 kg of peat soil. Soil with Chinese leek was called treated soil and soil without Chinese leek piece was regarded as untreated control. Treated and untreated soils were filled into 40-cm diameter and 50-cm deep pots, respectively, and one plantlet of Baxi (AAA) or Guangfen No.1 (ABB) planted per pot. Per treatment there were 20 pots and the experiment was replicated three times.

One week after planting, all plantlets were watered with a solution of spores of *Foc* race 4 to a total of 10,000 spores per g of soil. Leaf symptom assessments started ninety days after inoculation when the plantlets showed clear disease symptoms. Symptoms were recorded every 15 d until all the untreated control plantlets showed clear disease symptoms. Disease symptoms were assessed and incidence and severity index calculated as described above. To quantitatively measure the disease resistance, the Area under Disease Progress Curve (AUDPC) was calculated by the trapezoidal integration of the disease severity index from 90 to 180 days after inoculation as follows:

$$AUDPC=\sum_{i=1}^{n-1}\left[\frac{X_{i+1}+X_i}{2}\right](t_{i+1}-t_i)$$

Where X is the disease severity index, n the number of evaluations, and the (t_{i-1}-t_i) the time interval (days) between two consecutive evaluations (Das et al.1992)

Preparation of crude extract of Chinese leek

The aerial parts of the Chinese leek were ground into powder with liquid Nitrogen. 1,000 ml of sterilized water were added into 500 g of powder to leach the active ingredients for 24 h. The crude extract was concentrated to 50 ml using a rotary evaporator, and sterilized using a 0.22μm filter.

In vitro studies on solid media

PDA medium was prepared and sterilized by autoclaving at 121° for 15min. Crude extract of Chinese leek (0, 0.5, 1.0, and 2.0 ml, respectively) were added to Petri dishes and gently mixed with 18 ml PDA medium of about 50°. The final volume was supplemented to 20 ml with sterilized water. Immediately after the PDA medium solidified 10 μl of activated *Foc* race 4 cultures were inoculated onto the middle of the Petri dishes and the dishes were incubated at 28° in the dark. Evaluation of the inhibitory effect of the crude extracts on the mycelium growth of Foc race 4 was assessed by measuring the diameter of the fungal colonies after seven days. Each treatment was replicated six times.

In vitro studies on liquid media

Various amounts (0, 1, 5, and 10 ml) of crude extract of Chinese leek were added to a 100 ml Erlenmeyer flask. Two ml of activated *Foc* race 4 (10^6 spores/ml) labeled with green fluorescent protein were added to each flask, and PDA liquid media was added to a total volume of 20ml. The *Foc* race 4 spores and the crude extract were co-cultured on a shaking table at 150 rpm at 28° for 16h in dark. The growth status of the spores was detected with a Zeiss LSM510 laser scanning confocal microscope (Carl Zeiss, German) under white and fluorescent light. The number of the spores was counted in five visual fields (Plan-Neofluar 40× /1.3 oil DIC objective), the means are reported as the spore numbers in each treatment. The experiment was replicated three times.

Statistical analysis

Data were analyzed using SAS version 8.0 software. All data were checked for homogeneity of variance using “Levene” method before be submitted to one-way ANOVA analysis. The significance of the treatments was determined using Fisher’s protected least significant difference (LSD) test ($P \leq 0.05$).

Results

Incidence of *Fusarium* wilt in the field studies

The banana plants rotated with Chinese leek were generally healthy and few plants showed disease symptoms. In contrast, a great number of plants rotated with other crops were heavily infected and showed obvious yellowing and wilt symptoms (Fig.1). Over all three years, *Fusarium* wilt incidence and the disease severity index were significantly reduced by adopting the Chinese leek-banana rotation system ($P<0.0001$) (Table 1). In 2007, 132 of 7360 Chinese leek treated plants were infected and showed typical disease symptoms, while 2325 out of 5390 control plants rotated with Chinese quince or luffa had obvious disease symptoms. In 2008, 653 out of 23214 treated plants and 12469 of 42250 control plants (rotated with sugarcane) showed typical symptoms and in 2009, 1659 out of 39430 treated plants and 540 out of the 1500 control plants (rotated with rice) were infected. In the whole, the disease incidence of the treated plants was reduced by 96.6 % in 2007, 92.4 % in 2008, and 88.4 % in 2009. Moreover, the disease severity indices of the treated plants were reduced by 96.2 %, 94.1%, and 90.9% in 2007-2009, respectively, compared to those of the control plants. The mean yields per ha and the average crop value differed greatly between treated and control fields (Table 1). In the same market price, crop value was increased by 36.2 %-86.0 %.

Greenhouse inoculation experiment

Effects of Chinese leek on Fusarium wilt incidence in potted Baxi (AAA)—

Ninety days after inoculation, the treated plantlets and the untreated controls plantlets clearly differed in their disease symptoms (Fig.2 a, b). *Fusarium* wilt of the treated plantlets was significantly reduced by 65.2% compared to that of the untreated control plantlets (Fig 3). One hundred and fifty days after the artificial inoculation, 100% of the untreated control plantlets were infected and showed clear disease symptoms, but only 43.3% of the treated plantlets were infected. Disease severity index was 31.25 for the treated plantlets and 84.58 for the untreated control plantlets. Chinese leek reduced the disease severity index by 63.05%. Throughout the experiment, a significant difference existed between the treated plantlets and the untreated control plantlets on the disease incidence ($P<0.0001$), and the disease severity index ($P<0.0001$). Disease incidence and the disease severity index of the untreated control plantlets were higher than those of the treated plantlets by 58.06% and 61.82%, respectively (Fig 3). During the whole experiment, the AUDPC of the untreated control plantlets was 2.6 times higher than that of the treated plantlets ($P< 0.0001$) (Fig.3).

Effects of Chinese leek on the Fusarium wilt incidence in potted Guangfen No. 1 (ABB) in greenhouse—Guangfen No.1 (ABB) showed disease symptoms later than Baxi (AAA). Ninety days after inoculation (Fig. 2 c,d). *Fusarium* wilt incidence of treated Guangfen No.1 (ABB) plantlets was significantly reduced by 71.4 % compared with untreated control plantlets (Fig.3). One hundred and eighty days after artificial inoculation, 100% of the untreated control plantlets were infected and showed clear disease symptoms, but only 23.3% of the treated plantlets were infected. Final disease severity index was 10.83 for the treated plantlets and 70.42 for untreated control plantlets, a reduction of 84.6 %. Throughout the experiment, significant difference existed for the disease incidence ($P=0.0067$) and the disease severity index ($P=0.0062$) when comparing the treated and the untreated control plantlets. Disease incidence and disease severity index of the untreated control plantlets were higher than those of the treated plantlets by 79.15% and 80.67%, respectively (Fig.3). During the whole experiment, the AUDPC of the untreated control plantlets was 5.0 times higher than that of the treated plantlets ($P<0.0001$) (Fig.3).

In vitro studies

In solid media the crude extract of Chinese leek at 0.5, 1.0, and 2.0 ml per Petri dish significantly inhibited the mycelial growth of *Foc* race 4 by 12.1 %, 43.5 %, and 100 %, respectively (Table 2).

The crude extract of Chinese leek greatly suppressed the proliferation of *Foc* race 4 ($P<0.0001$) (Table 3. Fig. 4). Compared to the number of spores in the control under the white light, 1, 5, and 10 ml of Chinese leek crude extract suppressed the proliferation of the fungus by 79.2 %, 84.7 %, and 91.2 %, respectively. Comparing the number of spores under white light and the number of the spores under fluorescent light of the corresponding treatment shows that the crude extract of Chinese leek resulted in mortalities of *Foc* race 4 of 8, 53, and 87 % at 1, 5, and 10 ml Chinese leek crude extract per flask (Table 3). Differences were statistically significant when 5 ml or 10 ml of the crude extract of Chinese leek were added ($P=0.0135$ and $P=0.\ 0009$, respectively, Table 3).

Discussion

Overall, disease reductions in the field experiments were around 90 % and yield increases between 36 and 86 %. The field experiments were in line with farmer's experiences with the positive effects of Chinese leek in the rotation on *Fusarium* wilt.

Adoption of the rotation system has greatly reduced the *Fusarium* wilt incidence and has brought huge economic benefit to the farmers, which attracted more farmers to look for this kind of fields to plant bananas. So the rents of the fields where Chinese leek has been grown for years rose from about 20,000 to about 40,000 CNY (Chinese Yuan) ha^{-1} in recent years. Due to the shortage of this kind of Chinese leek field in the local area, some experienced farmers have moved to adjacent areas growing Chinese leek. As a result, the Chinese leek-banana rotation system has extended to other areas.

Our current field trials and the local farmers' experiences for more than ten years since 1997 show that the adoption of a Chinese leek-banana rotation system can be an efficient way to control *Fusarium* wilt in areas heavily infested with the pathogen. At present, in the Panyu area the following planting mode is very popular: a vegetable farmer rents fields and continuously cultivates Chinese leek for 3 years. Then a banana farmer rents the fields for another 3 years to plant Guangfen No.1 (ABB) for one crop, followed by another crop of Baxi (AAA). Afterwards the fields are transferred to a vegetable farmer again. In this way, *Fusarium* wilt is kept under control and, more importantly, the primary main banana cultivars can be produced again in this heavily *Foc* infected area. Thus, Chinese leeks have saved the banana production of the area.

In the laboratory, Chinese leek was highly effective in suppressing the growth and proliferation of *Foc* with a strong lethal effect. Growing Chinese leek in fields heavily infested by *Foc* apparently gradually reduces the population of *Foc*, to a low enough level to allow for successful banana production again. It is well known that some plant crude extracts possess antifungal activity (Ameziane *et al*. 2007; Bluma *et al*. 2008; Chaijuckam *et al*. 2010; Harish *et al*. 2008; Nguyen *et al*. 2009). Caraway and peppermint crude extracts cause complete growth inhibition of *Sclerotium rolfsii* (El-Mougy *et al*. 2009). Leaf crude extracts of *Abrus precatorius* and *Aegle marmelos* strongly inhibit *Colletotrichum capsici* and *Alternaria alternata* (Anand *et al*. 2009). *Salvia officinalis* crude extract controls grapevine downy mildew (Dagostin *et al*. 2010).

Antibacterial and antifungal effects of crude extracts of *Allium* plants have been studied extensively in food science (Najjaa et al.2007 , Mau et al. 2001, Lazarevic et al.2011, Yin et al. 1999) and medicine (Chen *et al*, 2011 , Seyfi. *et al* , 2010, Lee *et al*, 2004). There are also some reports on the effects on plant diseases. The crude extract of Green onion (*Allium fistulosum*), Garlic (*Allium sativum*) and Chinese leek (*Allium tuberosum*) inhibited the germination incidence of *Alternaria brassicicola* by 100% (Ho *et al*, 2007). Garlic (*Allium* sativum) crude extracts reduced disease incidence caused by *Pseudomonas syringae* pv.tomato (Pst) by 58%, and disease severity by 68 %(Balestra et al, 2009). A novel antifungal compound, fistulosim, isolated from Welsh onion (*Allium fistulosum* L.) showed high activity against *Fusarium* oxysporum (Phay *et al*, 1999. Our study proves again that *Allium* plants have antifungal effects.

Chinese leek is a common vegetable imposing no harm on humans and on the environment. So we believe that the use of Chinese leek to control *Fusarium* wilt has the potential to be an efficient, environmentally friendly way of disease management. We encourage the study and the application of this information to banana growers in various areas.

Acknowledgments

The research was supported by a grant from the National Science and Technical Support Project of P.R. China (2008BAD92B06) and the National "948" Project of P.R. China (2008-G1). We thank participating farmers for their contributions to this project, and Dr Altus Viljoen from University of Stellenbosch ,South Africa and Dr Uri Lavi from ARO-Volcani Center, Israel for suggesting advice for writing the paper.

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Figure 1.
Plantations of Guangfen No.1 (ABB) rotated with different crops in Panyu, Guangzhou, China in rotation with Chinese leek (a) rice (b), sugarcane (c) other vegetables (d). Symptoms in (b)-(d) are typical for *Fusarium* wilt.

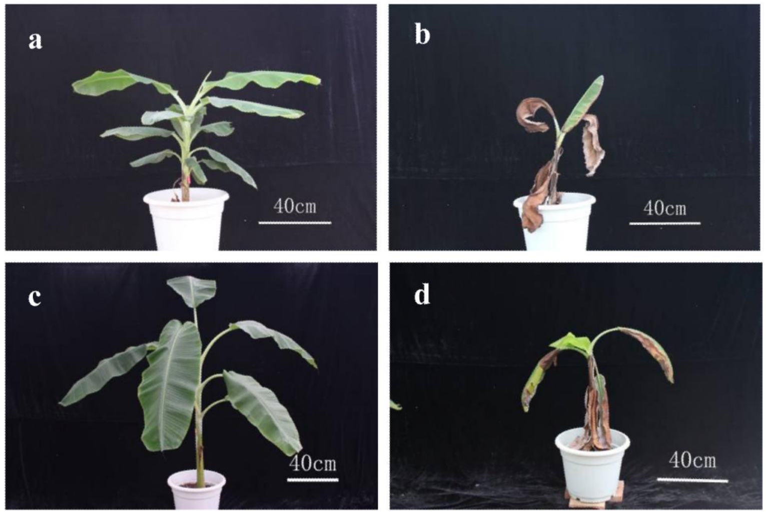

Figure 2.
Potted bananas 180 days after inoculation with *Foc* race4 in the greenhouse. Banana varieties Baxi (AAA) (a) and Guangfen NO.1 (ABB) (c) plantlets treated with Chinese leek developed in good health. The untreated control plantlets of Baxi (AAA) (b) and Guangfen NO.1 (ABB) (d) showed clear disease symptom.

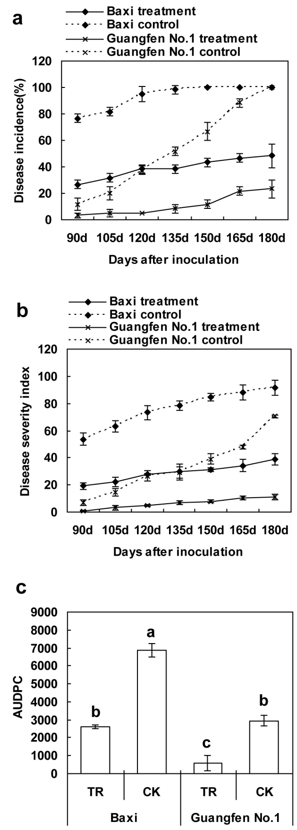

Figure 3.

Inhibitory effects of Chinese leek on Fusarium wilt incidence (a), disease severity index (b), and AUDPC(c) in potted banana plantlets inoculated with *Foc* race4. AUDPC was calculated from 90 to 180 days after inoculation(TR: Treatment, CK: Control). Values are the means of three replications with 20 plants in each treatment and vertical bars indicate the standard errors of the means.

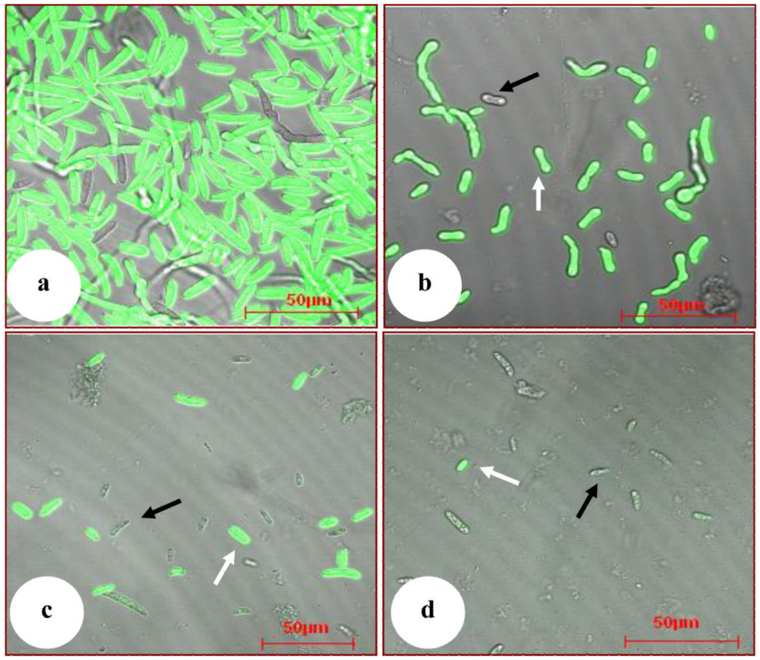

Figure 4.

Effect of crude extract of Chinese leek on the proliferation and fluorescence of spores of *Foc* race 4. Two ml of spores (10^6 spores/ml) of *Foc* race 4 labelled with green fluorescent protein were co-cultured with 0 ml (a), 1 ml (b), 5 ml (c), and 10 ml (d) of crude extract of Chinese leek leaves per 20 ml culture on a shaking table for 16 h, and then were detected with a Zeiss LSM510 laser scanning confocal microscope (Carl Zeiss, German) using a Plan-Neofluar 40× /1.3 oil DIC objective. Black arrows indicate dead spores without fluorescence reaction, white arrows indicate the live spores with fluorescence reaction.

Table 1

The means and corresponding standard error of Fusarium wilt incidence , disease severity index, yield per hectare and crop value of Guangfen No. 1(ABB) rotated with Chinese leek and other crops in Panyu, China between2007 and 2009.

Year	Treatment[y]	Disease incidence (%)	Disease severity index	yield (kg per ha)[y]	Crop value (CNY per ha)[z]
2007	CL	1.60±0.42	1.25±0.38	46125±200	276750±1205
	CK	47.03±7.45	37.42±6.58	24825±3490	148955±20941
P value		<0.0037	<0.0054	<0.0037	<0.0037
2008	CL	2.15±0.78	1.25±0.25	45867±367	275203±2205
	CK	28.18±2.62	21.58±1.69	33666±1225	201994±7355
P value		<0.0007	<0.0003	<0.0007	<0.0007
2009	CL	4.18±0.74	2.17±0.30	44916±347	269494±2086
	CK	35.93±2.33	24.58±2.06	30033±1093	180197±6558
P value		<0.0002	<0.0004	<0.00022	<0.0002

[y] CL means Guangfen No.1 rotated with Chinese leek (bananas plants grown in the fields where Chinese leek had been grown for three years), CK means bananas rotated with other crops, such as other vegetables(2007), sugarcane(2008) and rice(2009).

[z] Crop values based on market prices of 6.00 CNY(Chinese Yuan) per kg for Guangfen No.1 (ABB).

Table 2

The means and corresponding standard error of colony sizes of Foc race 4 on petri dish containing various quantities of the crude extracts of Chinese leek on 7 days after inoculation

Treatment	Fungi clone size(cm)[z]
0 uL(Control)	6.55±0.14a
500uL	5.75±0.19b
1000uL	3.70±0.18c
2000uL	0.00±0.00d
P Value	<0.0001

[z]The data were the means of six replications. Mean values in the same column followed by the different letter are significantly different by Fisher's protected least significant difference test (P<0.05).

Table 3

The means and corresponding standard error of the number of *Foc* race 4 spores labeled with green fluorescence protein detected with a laser scanning confocal microscope at 16h after co-culture with various quantities of crude extract of Chinese leek on a shaking table.

Treatment	The number of spores under white light[z]	The number of spores under fluorescent light[z]
0ml(Control)	142.7±6.69 a	142.7±6.69a
1ml	29.3±2.19 b	27.0±2.08b
5ml	21.7±2.03bc	10.3±1.76c
10ml	12.7±1.20c	1.7±0.33d
P Value	P<0.0001	P<0.0001

Values in the same column followed by the different letter are significantly different by Fisher's protected least significant difference test (P<0.05).