



Full Length Article

The Inhibitory Effect of the Plant Alkaloid Camptothecin on the Rice Sheath Blight Pathogen *Rhizoctonia solani*

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Abstract

The rice sheath blight caused by *Rhizoctonia solani* is one of the most destructive diseases of rice worldwide. Present study presented the inhibitory effect and mechanism of the plant alkaloid camptothecin on *R. solani*. Results indicated 14.32, 34.88, 59.94, 68.54, 76.38 and 77.72% inhibition of mycelial growth after 48 h of incubation at 6.25, 12.50, 25.00, 50.00, 100 and 200 µg/mL camptothecin. The soluble protein content in mycelia was significantly increased, the N-acetyl glucosamine content and chitinase activity was significantly reduced, but the sugar content was unaffected by camptothecin at 100 µg/mL after 12 h and 24 h treatment. Moreover, the optical microscopic and transmission electron microscopic observation revealed that the fungal mycelia are sparse with a loose structure, mitochondrial number, and morphological characteristics of camptothecin-treated *R. solani*. Overall, the antifungal activity of camptothecin against *R. solani*, suggested that camptothecin has a great potential in the control of rice sheath blight. © 2017 Friends Science Publishers

Keywords: Camptothecin; Antifungal activity; Mechanism; Antifungal; *Rhizoctonia solani*

Introduction

Rice sheath blight, is one of the most destructive diseases of rice worldwide, caused by *Rhizoctonia solani* Kühn (Rush and Lee, 1992). In highly intensive production systems in the China, rice sheath blight disease can cause yield and quality losses (Padaria and Singh, 2009). *R. solani* is also destructive for other economic crops such as corn (*Zea mays*) and soybean (*Glycine max*) (Ciampi *et al.*, 2008; Akhtar *et al.*, 2009). Using chemical fungicide can effectively control the severity of *R. solani*, however, chemical fungicide is generally considered to be among the potential health and environmental risks (Wang *et al.*, 2013). Hence, to reduce the harmful effects to the environmental search for novel natural product-based fungicide, has gained attention in the past decades (Xiong *et al.*, 2013).

In recent years, high biological activity on non-target organisms safety, environmental compatibility and good bio-synthetic pesticides has become a new trend in pesticide development. Botanical pesticides as part of the bio-synthetic pesticides, with its low toxicity, high selectivity, easy degradation and other unique advantages gained new opportunities for development. For research and development of botanical pesticides plant diseases become a hot research field of pesticides (Yang *et al.*, 2013).

Camptothecin (CPT), a modified monoterpene indole alkaloid, was isolated for the first time from *Camptotheca acuminata* (Sirikantaramas *et al.*, 2008). CPT has a planar pentacyclic ring structure, that includes a pyrrolo-quinoline moiety, conjugated pyridone moiety and one chiral center at position 20 within the α -hydroxy lactone ring with configuration (Reinke *et al.*, 2010). Interestingly, the CPT has been used worldwide for treating various kinds of cancers (Thomas *et al.*, 2004). Among the classical cytotoxic agents, CPT has been regarded as an important class of drugs with activity in tumor preclinical models (Vogel *et al.*, 2005). In particular, some CPT analogs may receive approval as anti-cancer drugs in the future (Sánchez-Ferrer *et al.*, 1995). Many studies have indicated that the antitumor effects of CPT should be attributed to the specific inhibition of eukaryotic DNA topoisomerase-I (topo-I), an enzyme playing pivotal roles in DNA replication, transcription, recombination, and repair (Fukuoka *et al.*, 1992; Subramaniana *et al.*, 2011). In addition to the low toxicity to human and animals, the use of the natural products CPT usually cause less environmental contamination and resistance than that of synthetic fungicides (Hsiang *et al.*, 1985; Lerchen and Baumgarten, 2001), indicating that CPT has great potential to be applied in agriculture. However, the successful development of an

effective fungicide from the natural products is still lacking.

The aim of this study was to examine the antifungal effect of CPT against rice sheath blight pathogen *R. solani* on the basis of morphological development, transmission electron microscopic observation and physiological change.

Materials and Methods

CPT Solutions and Fungus

CPT ($\geq 95\%$ pure) was purchased from Chengdu Furunde Industry Co., Ltd, China, while the solutions of CPT at different concentrations 6.25, 12.50, 25.00, 50.00, 100 and 200 $\mu\text{g/mL}$ were prepared by dissolving CPT in dimethyl sulfoxide (DMSO). Furthermore, *R. solani* used in this study were obtained from the school of Nanjing forestry university, China.

Inhibition of CPT against *R. solani*

A mycelial plug of *R. solani* was inoculated in the center of PDA medium containing Petriplate. After incubation for 48 h at 28°C, mycelial plugs (5.0 mm-diameter) from the margin of this fungal cultures were transferred to the new PDA media with the concentration of 0, 50, 100 and 200 $\mu\text{g/mL}$ CPT, respectively. PDA media with the same volume DMSO were used as the control. All plates were incubated at 28°C until the control plates were fully covered by the mycelium of *R. solani*. The mycelial diameter was recorded.

Optical Microscopic Observation

After incubation of 48 h at 28°C as described above, mycelia of *R. solani* from PDA media with CPT of 100 $\mu\text{g/mL}$ were picked up and observed for morphological development under Fluorescence differential microscope (Nikon, E50i, Japan). Photograph of mycelia from the control PDA medium without CPT was used as the control.

Effect CPT on Physiological Change

Plugs of 5.0 mm diameter were picked up from the PDA plates of *R. solani* and put into 50 mL Czapek broth: sucrose 30 g, NaNO_3 3 g, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 0.5 g, KCl 0.5 g, $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ 0.01 g, K_2HPO_4 1 g, Agar 13 g, distilled water 1000 mL, pH 7.2 with CPT at the final concentration of 100 $\mu\text{g/mL}$. The broths were shaken up at 120 rpm/min at 28°C. After 12 and 24 h, mycelia extracted by the filter of SHB-III vacuum pump (Yuhua, SHB-III, China), and washing 5 times using ultrapure water. Following air dry, mycelia were ground for 10 min by mixing them with quartz sands and 0.05 mol/L Tris - HCl solution (pH = 7.5) with a proportion of 1:5 (V: W) in an ice-bath mortar. After centrifugation at 12000 rpm for 20 min at 4°C, supernatant liquid were collected and stored at -20°C for further analysis. By the use of a visible spectrophotometer (Shimadzu, UV-2450,

Japan), the reducing sugar content in mycelia was measured as described by Su *et al.* (Su *et al.*, 2009) by determining the absorbance value at 540 nm wavelength. The soluble protein content, the N-acetyl glucosamine content, and the activity of chitinase were examined as described by Bradford (Bradford, 1979) and Li *et al.* (2000) by determining the absorbance at the wavelength of 595, 544 and 544 nm, respectively. Each sample had three replicates and the experiment was repeated twice.

Transmission Electron Microscopic Observation

After incubation at 28°C for 2 d, mycelia of *R. solani* were taken from the edge of media with and without CPT, respectively. Sample preparation and transmission electron microscopic observation was performed accord to the method of Li *et al.* (2013).

Statistical Analysis

The software STATGRAPHICS Plus, version 4.0 (Copyright manugistics Inc., Rockville, Md., USA) was used to perform the statistical analyses. Levels of significance ($P < 0.05$) of main treatments and their interactions were calculated by analysis of variance after testing for normality and variance homogeneity.

Results

Inhibition of CPT on Mycelial Growth

In the absence of CPT, the growth of *R. solani* strain ZJ001 increased with the increase of incubation time, while the diameter of the mycelium was 39.69 mm after 48 h of incubation. However, the mycelial growth was significantly inhibited by CPT at different concentrations. Indeed, the addition of CPT at the concentration of 6.25, 12.50, 25.00, 50.00, 100 and 200 $\mu\text{g/mL}$ resulted in a 14.32, 34.88, 59.94, 68.54, 76.38 and 77.72% inhibition in the mycelial growth of *R. solani* strain ZJ001 compared to the control, respectively (Fig. 1). Overall, this study revealed the in vitro antifungal activity of CPT against fungal pathogen *R. solani*, suggesting that CPT has a potential in controlling sheath blight of rice.

In agreement with the result, quite a few researches have focused on the antimicrobial activity of natural product CPT, which has been widely applied throughout the world for the treatment of various cancers (; Pucci *et al.*, 2016). Indeed, the growth inhibition of CPT against *Rhizoctonia cerealis*, *Sphaerotheca fuliginea* and *Pseudoperonospora cubensis* has been reported in our previous study, indicating that CPT had a great potential in agriculture. However, the successful development of an effective CPT fungicide is lacking, which make it very necessary to understand the mode of action of CPT against plant pathogens.

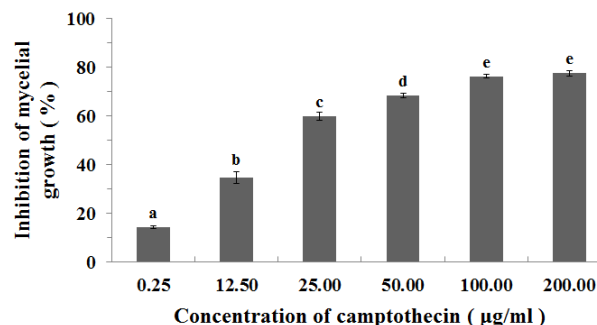


Fig. 1: Inhibitory effect of camptothecin at different concentration on the mycelial growth of *R. solani*

Optical Microscopic Observation of Mycelium

In addition to the reduction in mycelial diameter, the addition of CPT at 100 µg/mL also resulted in a change in mycelial morphology of *R. solani* strain ZJ001 compared to the control based on the optical microscopic observation. In the absence of CPT, the mycelium of *R. solani* grows rapidly in compact form and completely covers the PDA medium after 2 d incubation. However, in the presence of CPT, the mycelium of *R. solani* grew slowly in PDA medium compared to the control. In addition, the fungal mycelia are sparse with a loose structure (Fig. 2).

Effect of CPT on Physiological Properties

Result from this study indicated that treatment of CPT at 100 µg/mL was able to result in a significant change in physiological properties such as the reducing sugar content, soluble protein content, chitinase activity and N-acetyl glucosamine content in mycelium of *R. solani* strain ZJ001. These physiological parameters have been reported to be important for the structure and activity of fungal mycelium, which may at least partially explain the inhibition of CPT on the mycelial growth of *R. solani* strain ZJ001.

Result from this study indicated that the content of reducing sugar of *R. solani* mycelium was unaffected by CPT of 100 µg/mL after 12 h and 24 h of treatment. The soluble protein content of mycelium was significantly increased by CPT regardless of the treatment time. Indeed, CPT caused a 16.34 and 81.07% increase in the soluble protein content after 12 h and 24 h of treatment, compared to the corresponding control, respectively. The chitinase activity of mycelium was significantly reduced by CPT regardless of the treatment time. Indeed, CPT caused a 12.20 and 36.73% reduction in the chitinase activity after 12 h and 24 h of treatment, compared to the corresponding control, respectively. The N-acetyl glucosamine content of mycelium was significantly reduced by CPT regardless of the treatment time. Indeed, CPT caused a 18.60 and 16.05% reduction in the N-acetyl glucosamine content after 12 h and 24 h of treatment, compared to the corresponding control,

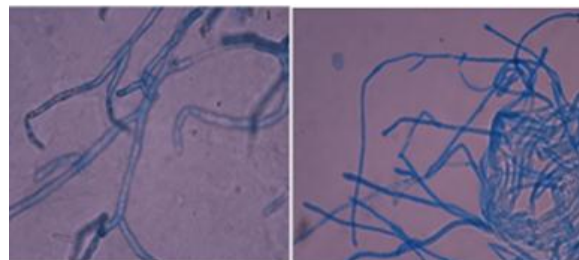


Fig. 2: Optical microscopic observation of mycelium 1: camptothecin; 2: Control

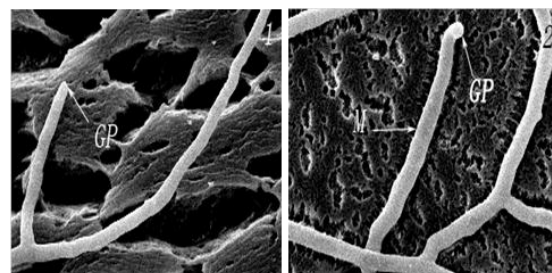


Fig. 3: Effect of camptothecin on the mycelial morphology of *R. solani* by transmission electron microscopic observation. GP: Growing point; M: Mycelium. 1: Control (500 ×); 2: Camptothecin (500 ×)

respectively (Table 1).

In agreement with the result the optical microscopic observation, this result indicated that CPT was able to cause a differential change in the four physiological parameters of *R. solani* mycelium, while the change was affected by both the kind of parameters other than the treatment time. Interestingly, infections of *R. solani* to rice plants were mainly due to the mycelia, emphasizing the importance of mycelia in this fungal pathogenicity. Therefore, this result from this study is not only is helpful for the understanding of antifungal mechanism of CPT against *R. solani*, but also justify the potential application in control of rice sheath blight caused by *R. solani*.

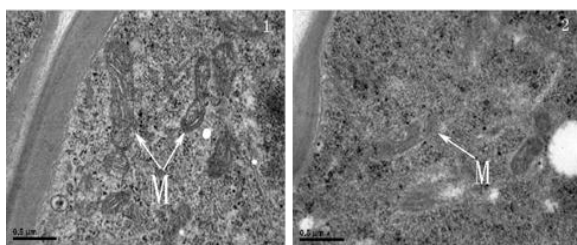
Transmission Electron Microscopic of Mycelium

Result from this study indicated that in the absence of CPT, the mycelia of *R. solani* strain ZJ001 with smooth surface were well-distributed. Furthermore, the growing point of mycelia is smooth and satiation, while the top of growing point is round based on transmission electron microscopic observation (Fig. 3). In addition, the normal mitochondria with clear cristae were observed in cells of *R. solani* strain ZJ001 (Fig. 4). However, in the presence of CPT, the growing point of mycelia became rough. Furthermore, the malformed and abnormal growing point of restrained and even stop the growth of mycelia (Fig. 3). In addition, the mitochondrial cristae became fuzzy, while the number of

Table 1: Effect of camptothecin (CPT) on the physiological activity of *R. solani* strain ZJ001

Parameters	OD values	
	12 h	24 h
Sugar content		
- CPT	0.125 ± 0.007 a	0.101 ± 0.005 a
+ CPT	0.120 ± 0.006 a	0.102 ± 0.006 a
Soluble protein		
- CPT	0.257 ± 0.013 b	0.243 ± 0.014 b
+ CPT	0.299 ± 0.016 a	0.440 ± 0.025 a
Chitinase		
- CPT	0.123 ± 0.008 a	0.049 ± 0.003 a
+ CPT	0.108 ± 0.005 b	0.031 ± 0.002 b
N-acetyl glucosamine		
- CPT	0.172 ± 0.009 a	0.081 ± 0.005 a
+ CPT	0.140 ± 0.007 b	0.068 ± 0.004 b

The concentration of CPT was 100 µg/mL

**Fig. 4:** Effect of camptothecin on the mitochondria morphology of *R. solani* by transmission electron microscopic observation. M: Mitochondria. 1: Control (50000 ×); 2: Camptothecin (50000 ×)

mitochondria was reduced by CPT in each cell of *R. solani* strain ZJ001 (Fig. 4).

Discussion

In general, transmission electron microscopic observation clearly revealed that the mycelial of *R. solani* strain ZJ001 was affected by CPT, which is consistent with the result of optical microscopic observation and measurement of physiological properties. Furthermore, in our previous study, the mycelial growth inhibition of *R. solani* has been mainly attributed to the antifungal mechanism of chitosan against the rice sheath blight (Liu *et al.*, 2012). In addition, CPT treatment resulted in the change in numbers and morphology of mitochondria, considering as the main site of respiration and “power source”. Obviously, this change in mitochondria can weaken the cell's breathing, resulting into the reduction in pathogenicity of this fungal pathogen. Therefore, this study indicated that CPT has a potential in controlling rice sheath blight caused by *R. solani*.

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