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Efficacy of *Rheum officinale* liquid formulation on cucumber powdery mildew

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ABSTRACT

Rheum officinale liquid formulation, the ethanol extract from roots of *R. officinale* Baill., formulated as physcion 5 g l^{-1} aqueous solution (AS), has been commercialized in China for controlling cucumber powdery mildew (*Podosphaera xanthii* (Castagne) U. Braun & Shishkoff). The efficacy of the product was evaluated in pot tests under controlled conditions and in open and protected fields in China over 2 years. In most trials, the efficacy reached above 80% at the rates of 10–50 mg a.i l^{-1} water after three applications and at the rates of 20–50 mg a.i l^{-1} water after two applications. The cucumber fruit yield in the product treatment was as many or more as triadimefon treatment, and over 53.1% compared to the untreated control. These results showed that Chinese rhubarb extract could be an effective alternative plant protecting agent in the integrated and biological management of cucumber powdery mildew.

1. Introduction

Powdery mildew caused by *Podosphaera xanthii* (Schlechtend.: Fr.) Pollacci is a widespread disease of greenhouse and field-grown cucumber plants and has been responsible for a large yield losses (Reuveni et al., 1996). Even though plant breeders have developed resistant cultivars against plant pathogen, it is not anticipated that genetic resistance will eliminate the need for fungicides (McGrath et al., 1996). Applying synthetic fungicides to control plant diseases has resulted in environmental pollution, phyto-toxicity to the host and a buildup in tolerance in plant pathogens to fungicides (McGrath et al., 1996; Sierotzki et al., 2000; Ishii et al., 2001). The use of plant extracts to control plant diseases is an alternative method to control the disease (Daayf et al., 1995; Choi et al., 2004; Kim et al., 2004). Besides their bioactivity against a range of target plant pathogens, plant extracts are biodegradable to nontoxic products, so are potentially suitable for use in integrated management programs, and could lead to the development of a new class of disease control agents (Balandrin et al., 1985).

The extracts from roots of rhubarb (including *Rheum officinale* Baill., *Rheum palmatum* L., *Rheum tanguticum* Maxim Regel, *Rheum emodi* Wall., *Rumex crispus* L., *Rheum laciniatum* Prain, etc.) were used to protect cucumber, wheat and barley from powdery mildew in controlled greenhouse and open field conditions (Tang et al., 2003; Choi et al., 2004; Yu et al., 2004, 2006; Yang, 2007). Besides powdery mildew, the extracts of rhubarb were reported to effectively control a number of plant diseases such as potato late blight (Jiang et al., 2001), tomato gray mould, wheat leaf rust, rice blast (Choi et al., 2004), strawberry root rot (Jiang and Lian, 2005), pepper mosaic disease and tomato mosaic disease (Zhu and Chiu, 1989; Guo et al., 1998).

The ethanol extract from roots of *R. officinale*, formulated as physcion 5 g l^{-1} aqueous solution (AS, Beijing Kingbo Biotechnology Co. Ltd., P.R. China), was commercialized for controlling cucumber powdery mildew in 2007. The objectives of this study were (1) to assess the level of control by *R. officinale* liquid formulation, (2) to measure the impact on yield, and (3) to determine suitable application rates of the product.

2. Materials and methods

2.1. Treatments

R. officinale liquid formulation, as physcion 5 g l^{-1} AS, was applied at the rates of 6.25, 10, 20 and 50 mg a.i l^{-1} water and the local standard fungicide 15% triadimefon wettable powder (WP) produced by Jianhu Agrochemical Co. Ltd. applied at the rate of 150 mg a.i l^{-1} water.

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Table 1
Effect of pot test of *Rheum officinale* liquid formulation and fungicide against cucumber powdery mildew in greenhouse.

Treatment	Rate (mg a.i l^{-1} water)	Colony no. per plant[a]	
		1st test	2nd test
Rheum officinale liquid formulation (Physcion 5 g l^{-1} AS)	6.25	24.7b	19.3b
	10	13.4a	12.7b
	20	9.5a	7.3a
	50	4.3a	4.7a
Triadimefon 15% WP	150	9.4a	7.0a
Untreated control	–	128.8c	104.3c

[a] Each value is an average of 4 replications. Means followed by the same letter are not significantly different at level 0.05 according to Duncan's test.

2.2. Pot tests

The pathogen used in the pot test was isolated from infected leaves of cucumber plants in Wuhan and reproduced on cucumber seedlings by Yang's method (Yang et al., 2007a). The pathogen was identified as *P. xanthii* based on microscopic characteristics of conidial germ tubes and chasmothecia (Zarakovitis, 1965).

The seedlings of cucumber cv Changchunmici (bought from Jingchu Seed Company) were grown in pots (400 mm in diameter), five plants a pot, in a controlled greenhouse (18–25 °C). At the four to five true leaf stage, *R. officinale* liquid formulation and triadimefon 15% WP was foliarly applied by a hand sprayer to run-off. The control plants were treated with water.

After being air-dried, the conidium suspension of 30 ml (containing 2×10^5 spore ml^{-1} of distilled water) was uniformly sprayed onto the leaves by a hand sprayer. The efficacy was assessed 10 d after treatment by counting the number of colonies on the leaves. The experiments were arranged in a completely randomized design with four replicates and repeated once.

2.3. Field trials

Six field trials were conducted in open fields and in a protected field in Inner Mongolia, Hubei, and Beijing during 2005–2006. The applications were designed as two to three times according to the local practice and the application interval was 7–10 d depending on the disease development in each field. The first application was conducted before or at the appearance of initial symptoms of powdery mildew. The fungicides were sprayed by a knapsack sprayer (Weishi® WS-16) delivering 900 l water ha^{-1} at 0.75 l min^{-1}. Plants were sprayed with water as untreated control. Disease severities were evaluated from 10 middle plants and four upper leaves for each plant. Before application, the disease severities were investigated for all the plots. Disease severity was recorded 7 or 14 d after the last application with the following scale: 0 = no colony, 1 = 0–5%, 3 = 6–15%, 5 = 16–25%, 7 = 26–50%, and 9 = more than 51% of leaf surface covered with mildew. The disease index (DI) for each treatment was calculated using the following formula:

$$\mathrm{DI} = \left(\sum_{i=0}^{9} Ni \times i\right) \Big/ \sum_{i=0}^{9} Ni/9 \times 100$$

where i indicates disease severity (0–9), Ni indicates the number of leaves with the severity of i.

The disease control (DC) was calculated as follows:

$$\%\mathrm{DC} = 100 \times [1 - (\mathrm{DI}_{\mathrm{UTC0}} \times \mathrm{DI}_{\mathrm{PT1}})/(\mathrm{DI}_{\mathrm{UTC1}} \times \mathrm{DI}_{\mathrm{PT0}})]$$

where UTC0 indicates the disease severity of the untreated control before the first treatment, UTC1 indicates the disease severity of the untreated control after treatment, PT0 indicates the disease severity of any treatment before the first treatment, PT1 indicates the disease severity of any treatment after treatment.

All the experimental sites were naturally infected and all cucumber cultivars were susceptible to powdery mildew. The dominant powdery mildew population was *P. xanthii* identified by the method of Section 2.2 in all the trials. No other fungicides were applied to experimental plots. All the other management was done in accordance with standard farm practices. Each treatment consisted of four replicates with 40 plants per plot and a 1-m guard row between each plot.

2.3.1. Trials in Inner Mongolia in 2005 and 2006

In 2005, the trial site was located in Baosu village, Taipingzhuang county, Huhehaote City, in the north of China. Seeds of cucumber cv "Xinshiji No. 2" (bought from Huhehaote Seed Company) were sown on May 28 and transplanted to an open field on June 18. *R. officinale* liquid formulation and triadimefon were applied weekly from July 22 three times. From July 22 to August 18 cucumber fruits were harvested 10 times at intervals of 2–5 d.

In the 2006 trial site located in Qianbuta village, Saihan town, Huhehaote City, north of China, the same cucumber cultivar was sown on May 18 and transplanted to an open field on June 18. *R. officinale* liquid formulation and triadimefon were first applied on July 8, and 7 d later (July 15) applied again. From July 8 to July 29 cucumber fruits were harvested 10 times at regular intervals of 2–4 d.

2.3.2. Trials in Hubei in 2005 and 2006

In 2005, the trial location was in the Vegetable Experimental Garden, Hongshan district, Wuhan city, central China. Seeds of cucumber cv "Xintaimici" (bought from Jingchu Seed Company) were sown directly in a controlled field on April 10. *R. officinale* liquid formulation and triadimefon were applied on May 20, and 7 d later (May 27) applied again. Between 15 May and 12 June cucumber fruits were harvested eight times at regular intervals of 2–5 d.

In 2006, the trial location was in the suburban of Shihua town of Gucheng county, north of Hubei provinces. The same cucumber cv was sown on March 25, transplanted to an open field on April 17. *R. officinale* liquid formulation and triadimefon were applied on

Table 2
Efficacy of *Rheum officinale* liquid formulation and fungicide against cucumber powdery mildew in an open field in Inner Mongolia in 2005.

Treatment	Rate (mg a.i l^{-1} water)	Disease index[a]			
		Before 1st application	7 d after 1st application	7 d after 2nd application	14 d after 3rd application
Rheum officinale liquid formulation (Physcion 5 g l^{-1} AS)	6.25	1.0a	1.2b	2.6c	7.6d
	10	0.9a	0.8a	1.7b	3.6c
	20	0.9a	0.7a	1.3ab	2.3b
	50	1.0a	0.6a	1.0a	1.3a
Triadimefon 15% WP	150	0.8a	0.7a	1.4ab	2.2b
Untreated control	–	1.0a	3.4c	7.2d	25.3e

[a] Each value is an average of results from 4 replications. Means followed by the same letter are not significantly different at level 0.05 according to Duncan's test.

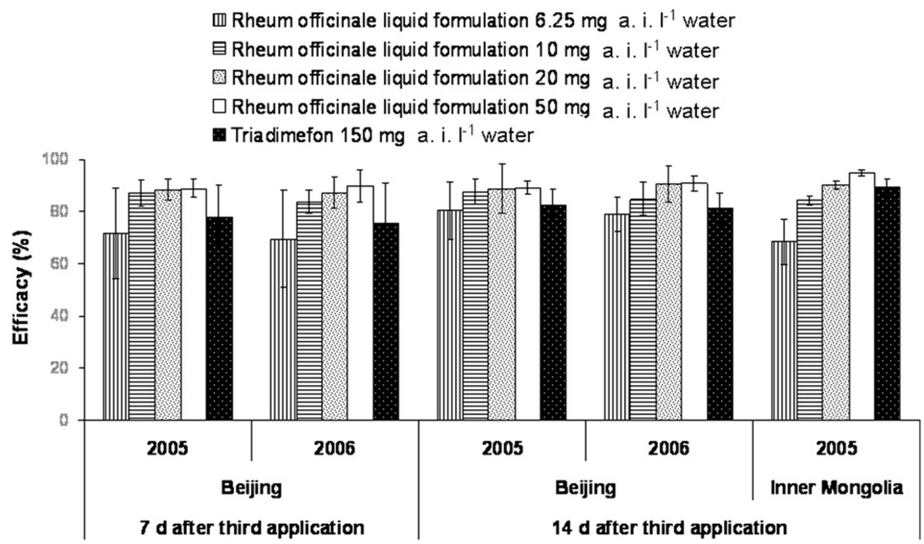

Fig. 1. Efficacy of *Rheum officinale* liquid formulation against cucumber powdery mildew after three applications. The disease indices of the untreated control were 35.4 in 2005 and 33.4 in 2006 in Beijing 7 d after three applications, 44.9 in 2005 and 51.5 in 2006 in Beijing and 25.3 in 2005 in Inner Mongolia 14 d after three applications. Data for each treatment are the means of four replicates. Vertical error bars represent standard errors of differences of means.

May 7, and 5 d later (May 12) applied again. Between 20 April and 22 May cucumber fruits were harvested 8 times at regular intervals of 2–5 d.

2.3.3. Trials in Beijing in 2005 and 2006

In 2005, the trial location was in Jingliyuan village, Miyun county, Beijing city. Seeds of cucumber cv "Jinyou No. 2" (bought from Institute of Cucumber Breeding of Tianjin) were sown on April 20 and transplanted to a controlled field on May 10. *R. officinale* liquid formulation and triadimefon were applied weekly from June 7 three times.

In 2006, the trial was in same location. The same cucumber cv was sown on April 10 and transplanted to a controlled field on April 29. *R. officinale* liquid formulation and triadimefon were first applied on May 22, 7 d later (May 29), and 14 d later (June 5) applied again.

3. Results

3.1. Pot tests

Results from the pot tests showed that the *R. officinale* liquid formulation had the excellent efficacy against powdery mildew. It provided equal efficacy to the local standard triadimefon at rate of 150 mg a.i l^{-1} water when applied at the rate of 10, 20 and 50 mg a.i l^{-1} water in the first test and at the rate of 20 and 50 mg a.i l^{-1} in the second test (Table 1).

3.2. Field trials

3.2.1. Inner Mongolia trials

In the trial of 2005, at the second and third evaluation disease on the untreated control developed slowly with disease severity of 3.4 and 7.2, respectively. At the last evaluation the disease had increased sharply with a disease severity of 25.3. All the treatments of *R. officinale* liquid formulation, reduced disease severities significantly (Table 2). The *R. officinale* liquid formulation at the rates of 20 and 50 mg a.i l^{-1} water provided equal or better control efficacy to that of 15% triadimefon WP at 150 mg a.i l^{-1} water (Fig. 1).

In 2006, the disease of the untreated control developed rapidly with disease severity of 20.6 at the second evaluation and 34.7 at the third. Treatments with *R. officinale* liquid formulation, reduced disease severity ($P < 0.05$) by Duncan's test (Table 3). The product, at the rates of 20 and 50 mg a.i l^{-1} water, provided equal control efficacy to that of the local fungicide treatment at 150 mg a.i l^{-1} water (Fig. 2).

3.2.2. Hubei trials

Similar results were obtained in 2005 and 2006. Powdery mildew severity on leaves of the untreated control reached 27.8 in 2005 and 18.9 in 2006 at the last evaluation. The disease severity was reduced significantly on leaves treated with *R. officinale* liquid formulation (Table 4). The product at 20 mg a.i l^{-1} water provided equal control efficacy to that of triadimefon at 150 mg a.i l^{-1} water, and at 50 mg a.i l^{-1} water, better than that of the local fungicide treatment (Fig. 2).

3.2.3. Beijing trials

There were very high disease pressures in both 2005 and 2006. The first application started before the appearance of powdery mildew in 2005. Powdery mildew severity on leaves of the untreated control reached 35.4 and 44.9 at the second and third evaluation respectively. The severity reduced significantly on leaves treated with *R. officinale* liquid formulation (Table 5). The product at the rates of 10–50 mg a.i l^{-1} water provided efficacy above 80% as

Table 3
Efficacy of *Rheum officinale* liquid formulation and fungicide against cucumber powdery mildew in an open field in Inner Mongolia in 2006.

Treatment	Rate (mg a.i l^{-1} water)	Disease index[a]		
		Before 1st application	7 d after 2nd application	14 d after 2nd application
Rheum officinale	6.25	0.8a	5.2c	8.8d
liquid formulation	10	0.9a	3.3b	5.1c
(Physcion	20	0.8a	1.9a	2.8b
5 g l^{-1} AS)	50	0.7a	1.0a	1.0a
Triadimefon 15% WP	150	0.7a	1.6a	2.2ab
Untreated control	–	0.9a	20.6d	34.7e

[a] Each value is an average of results from 4 replications. Means followed by the same letter are not significantly different at level 0.05 according to the Duncan's test.

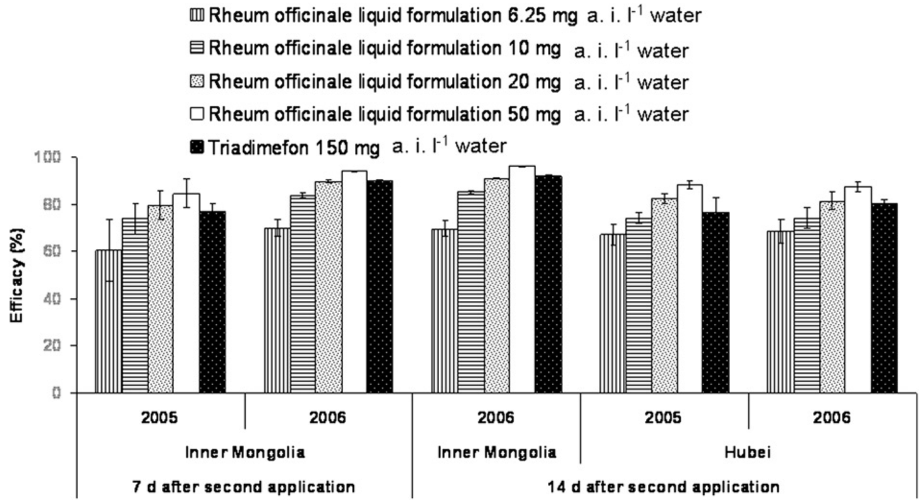

Fig. 2. Efficacy of *Rheum officinale* liquid formulation against cucumber powdery mildew after two applications. The disease indices of untreated control were 7.2 in 2005 and 20.6 in 2006 in Inner Mongolia 7 d after two applications, 27.8 in 2005 and 18.9 in 2006 in Hubei and 34.7 in 2006 in Inner Mongolia 14 d after two applications. Data for each treatment are the means of four replicates. Vertical error bars represent standard errors of differences of means.

triadimefon at 150 mg a.i l^{-1} water. Similar results were obtained in 2006 (Fig. 1).

3.2.4. Yield assessment

During 2005–2006, yield assessments were conducted in Hubei and Inner Mongolia. Treatments of *R. officinale* liquid formulation enhanced the fruit yield of cucumber by 13.1–53.1% at the rates of 10–50 mg a.i l^{-1} water; compared to the untreated control, the product at the rates of 10–20 mg a.i l^{-1} water, increased fruit mass per plant by more than 200.0 g as triadimefon at 150 mg a.i l^{-1} water in Inner Mongolia and Hubei. In all the trials, cucumber yield was significantly higher in the product treatment at the rate of 50 mg a.i l^{-1} water than in the local fungicide treatment (Table 6).

4. Discussion

R. officinale liquid formulation provided effective control of cucumber powdery mildew in the six field trials during 2005–2006. Cucumber yields increased significantly in all the product treatments compared with the untreated control. The recommended rates were suggested as 10–50 mg a.i l^{-1} water and the first application was conducted before or at the start of powdery mildew symptoms, with the next application at an interval of 7 d and from two to three applications.

R. officinale liquid formulation contains anthranqunone derivatives physcion, emodin, rhein, chrysophenol and aloe-emodin. Physcion is considered as the key active ingredient in the product (physcion 5 g l^{-1} AS) because its bioactivity is much better than those of other anthranqunone derivatives chrysophanol, emodin, rhein, etc. Moreover, there was a synergistic interaction between physcion and chrysophanol against the pathogen of powdery mildew (Yang, 2007; Yang et al., 2007b).

The methanol extract from the leaves of *Reynoutria sachalinensi* (F. Schmidit) Nakai, containing an active ingredient physcion, was commercialized as Milsana® by Compo GmbH and registered as plant strengthener in Germany. When applied as preventive treatments, the product gave satisfactory performance against powdery mildews on greenhouse crops (Daayf et al., 1995; Wurms et al., 1999; Petsikos-Panayotarou et al., 2002). Wurms et al. (1999) reported that Milsana® application on cucumber plants significantly reduced disease incidence of powdery mildew through inducing localized resistance, and microscopic observations showed that most haustoria collapsed in the localized Milsana® treatment. However, in our previous experiment, physcion could reduce the length of primary haustoria and the number of secondary haustoria, but no collapsed haustoria were observed in the barley/powdery mildew system after the treatment of physion (Yang et al., 2008a).

Studies showed that Milsana®-induced resistance of plants against pathogens was due to increased chlorophyll values and activities of POD and β-1,3-glucanase and ethylene (Herger and Klingauf, 1990). Daayf et al. (1997) reported that this product could induce disease resistance of cucumber plants by increasing the content of phenolics. Fofana et al. (2005) reported that the induced resistance of crops by the product could be suppressed by down regulating chalcone synthase, a key enzyme of the flavonoid pathway. Our recent research analyzed the differential gene profiles of physcion treatment and non-physcion treatment with Affymetix Genechip Microarray. Five down-regulated genes and four up-regulated genes were involved. Though the five down-

Table 4
Efficacy of *Rheum officinale* liquid formulation and fungicide against cucumber powdery mildew in controlled fields in 2005 and open field in 2006 in Hubei.

Treatment	Rate (mg a.i l^{-1} water)	Disease index 2005[a]		Disease index 2006[a]	
		Before 1st application	14 d after 2nd application	Before 1st application	14 d after 2nd application
Rheum officinale liquid formulation (Physcion 5 g l^{-1} AS)	6.25	1.5a[a]	9.9d	1.1a	6.6d
	10	1.4a	7.6c	1.1a	5.7c
	20	1.4a	5.0b	1.0a	3.5b
	50	1.4a	3.3a	1.0a	2.3a
Triadimefon 15% WP	150	1.3a	6.2b	1.1a	4.4b
Untreated control	–	1.4a	27.8e	1.0a	18.9e

[a] Each value is an average of results from 4 replications. Means followed by the same letter are not significantly different at level 0.05 according to Duncan's test.

Table 5
Efficacy of *Rheum officinale* liquid formulation and fungicide against powdery mildew in controlled fields in Beijing in 2005 and 2006.

Treatment	Rate (mg a.i l^{-1} water)	Disease index 2005[a]		Disease index 2006[a]	
		7 d after 3rd application	14 d after 3rd application	7 d after 3rd application	14 d after 3rd application
Rheum officinale liquid formulation (Physcion 5 g l^{-1} AS)	6.25	9.8a	8.6a	9.3a	10.7a
	10	4.8a	5.3a	5.3a	7.7a
	20	4.3a	5.0a	4.2a	5.2a
	50	4.0a	5.0a	3.5a	4.7a
Triadimefon 15% WP	150	7.5a	7.7a	7.5a	9.3a
Untreated control	–	35.4b	44.9b	33.4b	51.5b

[a] Each value is an average of 4 replications. Means followed by the same letter are not significantly different at level 0.05 according to Duncan's test.

Table 6
Yield assessment in Hubei and Inner Mongolia from 2005 to 2006.

Treatment	Rate (mg a.i l^{-1} water)	Inner Mongolia[a] (Weight of fruit $plant^{-1}$)		Hubei[a] (Weight of fruit $plant^{-1}$)	
		2005	2006	2005	2006
Rheum officinale liquid formulation (Physcion 5 g l^{-1} AS)	6.25	1000.0d[a]	918.8c	701.9d	711.5d
	10	1068.8c	1000.0b	750.0c	762.8cd
	20	1143.8b	1056.3ab	817.3b	820.5b
	50	1262.5a	1112.5a	868.6a	887.8a
Triadimefon 15% WP	150	1150.0b	1050.0ab	782.1bc	791.7bc
Untreated control	–	825.0e	781.3d	663.5e	653.8e

[a] Each value is an average of results from 4 replications. Means followed by the same letter are not significantly different at level 0.05 according to Duncan's test.

regulated genes were defence-related genes linked to POD, oxalate oxidase, bsil protein and pathogenesis-related protein, the up-regulated genes of barley were leaf-specific thionin. It is interesting that five probe sequences blasted the same gene and the changes were significant. The qRT-PCR data verified that the gene expression of leaf-specific thionin increased significantly when the barley leaves were treated with physcion (data not shown).

In past studies, physcion was selected to represent the active ingredients to establish baseline sensitivity of powdery mildew and downy mildew populations on cucumber. Baseline sensitivity was distributed as a normal unimodal curve with mean EC_{50} 0.304 mg l^{-1} for powdery mildew populations and mean EC_{50} 0.501 mg l^{-1} for downy mildew populations. The powdery mildew isolate did not decrease sensitivity under the selection pressure of physcion at the rate of EC_{70} for 15 generations. Plant pathogens *P. xanthii* and *Pseudoperonospora cubensis* showed a low resistance risk to physcion (Yang et al., 2008b).

According to the results of the past and present studies, *R. officinale* liquid formulation, physcion 5 g l^{-1} AS, has a low resistance risk and high control efficacy on powdery mildew, so it could be considered as a good alternative control measure in integrated cucumber disease management.

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