

WANG Shutong, HU Tongle, ZHANG Fengqiao, H. R. Forrer, CAO Keqiang

# Screening for plant extracts to control potato late blight

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**Abstract** Six extracts from plant material (*Galla chinensis*, *Potentilla erecta*, *Rheum rhabarbarum*, *Salviae officinalis*, *Sophora flavescens*, and *Terminalia chebula*) were tested for controlling effects against the infection of *Phytophthora infestans* on detached potato leaves, seedlings, and tuber slices. On detached leaves, *G. chinensis* (2%), *R. rhabarbarum* (rhizome, 2%) and *S. flavescens* (2%) extracts showed a significant control effect, with a control efficacy of 96.67%, *G. chinensis* was the best. On seedlings *R. rhabarbarum* (rhizome, 2%) showed the best inhibiting effect, followed by *S. flavescens* (2%), *T. chebula* (1%), and *G. chinensis* (2%). The control efficacies were 91.67%, 75.00%, 70.24%, and 64.29%, respectively on the seventh day after inoculation. However, on potato slices, none of the plant extracts showed effective protection against infection and sporangia production by *P. infestans*. The reason was analyzed and the potential for developing a natural fungicide based on these plant materials was discussed.

**Keywords** potato late blight, plant extracts, disease control

## 1 Introduction

*Phytophthora infestans* (Mont.) de Bary, the pathogen causing potato late blight and tomato late blight, is one of the

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WANG Shutong (✉)  
Bio-control Center of Plant Disease and Plant Pests of Hebei Province,  
College of Plant Protection, Agricultural University of Hebei, Baoding  
071001, China  
State Key Laboratory of Tropical Crop Biotechnology Institute of Bio-  
science and Biotechnology, Chinese Academy for Tropical Agricultural  
Sciences, Haikou 571101, China

HU Tongle, ZHANG Fengqiao, CAO Keqiang (✉)  
Bio-control Center of Plant Disease and Plant Pests of Hebei Province,  
College of Plant Protection, Agricultural University of Hebei, Baoding  
071001, China  
E-mail: ckq@hebau.edu.cn

H. R. Forrer (✉)  
Swiss Federal Research Station for Agroecology and Agriculture (FAL)  
Reckenholz, Zürich CH-8046, Switzerland

biggest problems in potato and tomato production. The disease is currently mainly controlled by growing resistant varieties or by spraying copper fungicides (Cao et al., 2001a). However, resistant varieties are rather rare and consumers often refuse to use them because of the poor processing characters. Copper fungicides contain copper, a heavy metal that has a wide range of side effects. Therefore, the use of copper in organic agriculture in the European Union is rather restricted and will be gradually banned in the near future.

The use of natural products for the control of fungal diseases in plants is considered as an interesting alternative to synthetic fungicides due to their less negative impacts on the environment (Cao and Forrer, 2001b). Dozens of plant extracts or plant essential oils have been tested against *P. infestans* *in vitro* for the inhibitory effect and the control efficacy under greenhouse conditions. Some plant materials, e.g., *Potentilla erecta* and *Salviae officinalis* showed a promising effect against potato late blight (Quintanilla et al., 2002; Blaeser and Steiner, 1999). In this paper, plant extracts from several Chinese traditional medicinal herbs were tested for controlling effects against potato late blight on detached potato leaves, seedlings and potato slices in the hope of finding such an alternative.

## 2 Materials and methods

### 2.1 Medicinal plants and the plant parts

Foliage of *Galla chinensis*, roots of *Potentilla erecta*, foliage and roots of *Rheum rhabarbarum*, foliage of *Salviae officinalis*, bark of *Sophora flavescens*, and fruits of *Terminalia chebula* were used.

### 2.2 Plant extracts

The prepared fine powder (0.75 mm in diameter) of 40 g of dried plant materials (DM) was macerated with 200 mL of ethanol (80%) for 24 h in 500 mL flasks and treated in an ultrasonic cleaner for 10 min. Then the mixtures were centrifuged at 4 000 r/min for 20 min. The supernatant was evaporated under reduced pressure at a temperature below 45°C. The residues were dissolved in 40 mL ethanol (50%) as

original extracts (OE) and stored at 5°C in darkness. The *Rheum rhabarbarum* plant materials (roots and leaves) were extracted separately.

## 2.3 Trials

### 2.3.1 Trial on detached potato leaves

Detached leaves were from potato plants (cv. Agria), four weeks after planting. Three compound leaves were put into a plastic box (32.5 cm × 21.5 cm × 2.5 cm) with two pieces of wet filter paper (18.5 cm in diameter). The boxes were used to maintain high humidity. Into each box, 10 mL distilled water was added. One milliliter original extract (OE) was added to 49 mL distilled water with the surfactant Tween-20 (0.0125%) to make a 2% solution. *Sophora flavescens* and *Terminalia chebula* extracts were tested at three concentrations (2%, 1%, and 0.5%). All the other extracts were tested at 2%. Within 30 minutes after preparation the solution was atomized onto the leaves in the plastic box until droplets became visible on the surface. Controls were treated with 50 mL distilled water with the surfactant Tween-20 (0.125 mL/L). Each treatment involved four replicates. All the boxes were incubated at 17°C in the dark. Sporangia suspensions of *P. infestans* ( $1 \times 10^5$  sporangia/mL) were inoculated onto the surface of the leaflets 24 h later. Three leaflets per leaf were inoculated. Afterwards, all the boxes were incubated at 17°C in the dark. Disease evaluation was conducted 7 days after inoculation. Disease incidence and disease index were assessed separately.

Disease severity was evaluated according to the following scales:

Assessment of late blight severity on potato leaves

0: No disease;

1: Small lesion on the inoculated point with the lesion area less than 10% of the whole leaflet;

3: Lesion area between 10% and 20% of whole leaflet;

5: Lesion area between 20% and 30% of whole leaflet, the waterish area less than 50% of the whole leaflet;

7: Lesion area between 30% and 60%;

9: Lesion area over 60% of the whole leaflet.

The disease incidence, disease index and control efficacy were calculated as follows:

Disease incidence (%) = (leaflets diseased/total number of leaflets inoculated) × 100,

Disease index =  $\sum(\text{disease severity level} \times \text{number of leaflets with the same severity level}) / (\text{total number of leaflets} \times 9) \times 100$ ,

Control efficacy (%) = (disease index of untreated control – disease index of treatment)/disease index of untreated control × 100.

### 2.3.2 Trial on seedlings

Potato tubers of cv. Agria were grown in the greenhouse. Four weeks after planting, Alar (85% Bernsteinsäure-2, 2-dimethylhydrazid) was applied to prevent the plants from

growing too high. For *Sophora flavescens* and *Terminalia chebula*, three concentrations (2%, 1%, and 0.5% of dry material [DM]) were used. The other plant materials were tested at a concentration of 2% (1 mL OE with 49 mL distilled water containing the surfactant Tween-20 [0.0125%]). Controls were treated with 50 mL distilled water with the surfactant Tween-20 (0.0125%). Within thirty minutes after preparation the solutions were atomized onto the potato leaves with a hand-held sprayer. Twenty-four hours later, 30 µL sporangia suspension of *P. infestans* (concentration approximately  $1 \times 10^5$  sporangia/mL) was inoculated on marked leaves. After inoculation, 100% relative humidity was maintained in the greenhouse for 24 h. Three leaflets were inoculated per plant, and four plants were used as four replications for each treatment. A randomized block design was used for this experiment. The disease incidence was determined 7 and 9 days after inoculation. Seven days after incubation (room temperature, 16/8 h day/night, and artificial illumination), the disease severity was evaluated and the disease index was calculated as the above mentioned.

### 2.3.3 Trial on tuber slices

Potato tubers of cv. Nicola were used in this trial. All the plant extracts were diluted to 2% (DM) with sterile water (800 µL extracts in 40 mL sterile water). Potato slices were completely submerged in these solutions for 3 min and then put into Petri dishes. Afterwards, these slices were incubated at 18°C for 24 h. Each potato slice was inoculated with 30 µL of a sporangia suspension *P. infestans* (concentration of  $1 \times 10^5$  sporangia/mL). Potato slices dipped into sterile water were used as control. The slices were incubated in the dark at 17°C for 10 days. Four replications were used for each treatment. The disease incidence was recorded and the quantity of the sporangia was counted by using a Thoma chamber (5 mL distilled water was used to harvest the sporangia on one potato slice; three samples were counted per slice and the three counts were averaged).

## 2.4 Statistical analyses

Each treatment was replicated four times in a randomized complete block design. All the trials were conducted twice. The data were subjected to analyses of variance (ANOVA) with the WIDAS Software package of MSI Wälti AG Buchs CH. Newman-Keul's ( $P = 0.05$ ) multiple range tests were used to compare the different treatments. In the greenhouse data, there was homogeneity of error variances, and the data for two repeated trials were combined.

## 3 Results

### 3.1 Trial on detached leaves

The control effect of the plant extracts on the infection of detached leaves of cv. Agria is shown in Table 1. According

**Table 1** Control effect of plant extracts on infection of detached potato leaves of cv. Agria by *P. infestans*

Treatment	Concentration/%	Disease incidence <sup>1</sup> /%	Disease index <sup>1</sup>	Control efficacy /%
Water with Tween-20	0.0125	50.00 cd	35.71 b	—
Untreated control	0	75.00 abc	53.57 a	—
<i>Salvia officinalis</i>	2	83.33 a	34.52 b	35.56
<i>Potentilla erecta</i>	2	79.17 ab	33.93 b	36.67
<i>Terminalia chebula</i>	0.5	62.50 abcd	31.55 b	41.11
<i>Terminalia chebula</i>	1	75.00 abc	30.95 b	42.22
<i>Sophora flavescens</i>	0.5	58.33 abcd	27.38 bc	48.89
<i>Rheum rhabarbarum</i>	2 (foliage)	54.17 bcd	26.19 bc	51.11
<i>Sophora flavescens</i>	1	45.83 d	17.86 cd	66.67
<i>Terminalia chebula</i>	2	62.50 abcd	16.07 cd	70.00
<i>Sophora flavescens</i>	2	50.00 cd	13.10 de	75.56
<i>Rheum rhabarbarum</i>	2 (roots)	45.83 d	10.12 de	81.11
<i>Galla chinensis</i>	2	4.17 e	1.79 e	96.67

<sup>1</sup>: Significance level of Newman-Keul's ( $P = 0.05$ ) multiple range tests. Numbers within a column followed by the same letter are not significantly different at  $P = 0.05$ .

to disease incidence, treatment with *G. chinensis* (2%) gave the best control against infection by *P. infestans*. *R. rhabarbarum* roots (2%), and *S. flavescens* (1%) were also effective. The other plant extracts did not have significant control effects, compared with the control treatments.

According to disease index, extracts of *G. chinensis* (2%), *R. rhabarbarum* roots (2%), *S. flavescens* (2% and 1%), and *T. chebula* (2%) had a significant control effect on infection by *P. infestans*. The control efficacies were 96.67%, 81.11%, 75.56%, and 70.00%, respectively.

### 3.2 Trial on seedlings

The control effect of the different plant extracts against infection of seedlings of cv. Agria by *P. infestans* is shown in Table 2. Seven days after inoculation, plant extracts of *G. chinensis*, *R. rhabarbarum*, *S. flavescens* and *T. chebula* significantly controlled the infection by *P. infestans*. The remarkable control effect was obtained with plant extracts of *G. chinensis*, *R. rhabarbarum* (roots), *S. flavescens* (2%) and *T. chebula* (1%).

Nine days after inoculation, only the plant extract of *R. rhabarbarum* (roots) significantly controlled the infection by *P. infestans* compared to both control treatments (untreated and water with Tween-20 [0.0125%]). Plant extracts of *G. chinensis*, *S. flavescens* (1%) and *T. chebula* (1% and 0.5%) showed a significant control effect only when compared with the untreated control.

### 3.3 Trial on tuber slices

The results on tuber slices are shown in Table 3. In this experiment, the extract of *T. chebula* was made from seeds and skins in addition to the whole fruits. Each part was tested separately. The extract of *S. flavescens* appeared to have the best controlling effect against the production of sporangia of *P. infestans*, though it was not significant, compared with the control. Some materials (such as extract of *T. chebula* seeds) seemed to promote the formation of sporangia of *P. infestans*. The fungus developed on each slice, showing that once the pathogen reached the inside of the tuber, it was extremely difficult to prevent the tuber from getting fully rotted.

**Table 2** Control effect of plant extracts on infection of *P. infestans* on potato seedling (cv. Agria)

Treatment	Concentration/%	7 days after inoculation		9 days after inoculation	
		Disease index <sup>1</sup>	Control efficacy/%	Disease index <sup>1</sup>	Control efficacy/%
Water with Tween-20	0.0125	53.70 bc	—	75.93 abc	—
Untreated control	0	77.78 a	—	100.00 a	—
<i>Potentilla erecta</i>	2	66.67 ab	14.29	88.89 ab	11.11
<i>Rheum rhabarbarum</i>	2 (foliage)	41.67 cd	46.43	69.44 abc	30.56
<i>Salvia officinalis</i>	2	40.74 cd	47.62	88.89 ab	11.11
<i>Sophora flavescens</i>	0.5	37.04 cd	52.38	87.04 ab	12.96
<i>Sophora flavescens</i>	1	36.11 cd	53.57	57.41 bcd	42.59
<i>Terminalia chebula</i>	2	31.48 cd	59.52	75.93 abc	24.07
<i>Terminalia chebula</i>	0.5	31.48 cd	59.52	60.19 bcd	39.81
<i>Galla chinensis</i>	2	27.78 de	64.29	60.19 bcd	39.81
<i>Terminalia chebula</i>	1	23.15 de	70.24	42.59 cd	57.41
<i>Sophora flavescens</i>	2	19.44 de	75.00	81.48 abc	18.52
<i>Rheum rhabarbarum</i>	2 (roots)	6.48 e	91.67	27.78 d	72.22

<sup>1</sup>: Significance level of Newman-Keul's ( $P = 0.05$ ) multiple range tests. Numbers within a column followed by the same letter are not significantly different at  $P = 0.05$ .

**Table 3** Effect of plant extracts on the sporangia concentration of *P. infestans* on potato slices of the cv. Nicola

Treatment	Concentration /%	Disease incidence <sup>1</sup> /%	Sporangia concentration / ( $\times 10^4$ sporangia $\cdot$ mL <sup>-1</sup> )
<i>Terminalia chebula</i>	2 (seed)	100	5.08 a
<i>Rheum rhabarbarum</i>	2 (foliage)	100	3.21 ab
<i>Galla chinensis</i>	2	100	2.79 bc
<i>Terminalia chebula</i>	2 (fruit)	100	2.25 bc
Untreated control	0	100	2.04 bc
<i>Potentilla erecta</i>	2	100	2.00 bc
<i>Salviae officinalis</i>	2	100	1.92 bc
<i>Rheum rhabarbarum</i>	2 (root)	100	1.46 bc
<i>Terminalia chebula</i>	2 (skin)	100	1.33 bc
<i>Sophora flavescens</i>	2	100	0.83 bc

<sup>1</sup>: Significance level of Newman-Keul's ( $P = 0.05$ ) multiple range tests. Numbers within a column followed by the same letter are not significantly different at  $P = 0.05$ .

## 4 Discussion

In China, alternative materials to the commonly used fungicides are being sought to control potato late blight in a more sustainable and environment-friendly way (Cao et al., 2001a). Most of the materials tested here were Chinese traditional medicines. For the trial on detached leaves, *G. chinensis*, *R. rhabarbarum*, *S. flavescens*, and *T. chebula* gave promising results, while the results differed somewhat according to disease incidence and disease index. The disease incidence results showed the effect of the extracts on primary infection of potato late blight. The disease index results were the integration of disease incidence and disease severity, and not only showed the control effect against primary infection, but also prevented late blight from spreading. For the trial on seedlings, the extract of *R. rhabarbarum* (roots) showed the best control effect in all the tested materials. While, none of the plant extracts tested was able to control the infection of *P. infestans* on potato tuber slices. The failure of the protection on slice could be caused by the decline of the defense system of the tuber after cutting. The extract of medicinal plants could not provide 100% protection. Once the potato slice lost its natural defense system, the fungus could soon cover the surface and produce much sporangia. That could explain why the protection effects on potato slices were so low.

*G. chinensis* is the hard, globular secretion on the leaves and stems induced by the larva of the aphid *Melaphis chinensis* (Hou et al., 2005). The extract from *G. chinensis* provided a significant controlling effect against the infection of *P. infestans* in trials on detached leaves and seedlings in the greenhouse 7 days after inoculation.

*S. flavescens* is a commonly used Chinese medicine (Li et al., 2004). Plant extracts of *S. flavescens* and *T. chebula* (Yang et al., 2004; Yang et al., 2003) significantly controlled the infection of *P. infestans* in trials on both the detached

leaves and seedlings in the greenhouse 7 days after inoculation. However, the control effect declined quickly 9 days after inoculation. The same situation happened on *G. chinensis*. The reason for the loss of the effect was nothing but the lesion development on leaf surface. Once the lesion emerged on the leaf surface, it sooner or later would damage the whole leaf. If the evaluation was made late, the disease index would increase greatly, with a result of a not so significant difference between the testing materials and untreated check.

*R. rhabarbarum* (medicinal rhubarb) is a popular Chinese medicine (Ding and Xu, 2004). In these trials, the root extract from *Rheum rhabarbarum* showed a significant control effect against infection of *P. infestans* on the detached leaves and the seedlings in the greenhouse. Of all the plant extracts tested, this one had the best controlling effect on the potato late pathogen, *P. infestans*.

In all the above tests, extracts from *R. rhabarbarum*, *G. chinensis*, *S. flavescens* and *T. chebula* seemed to have the potential as alternative materials for potato late blight control. It is advisable that field trials should be done before any affirmative conclusion is drawn.

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