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Controlling Phytophthora blight of pepper in Guizhou Province of China using *Stellera chamaejasme* extracts and synthetic chemical fungicides

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Abstract: Phytophthora blight of pepper, caused by *Phytophthora capsici* Leonian, is a destructive disease in pepper production. Extracts of the plant species *Stellera chamaejasme* Linn, previously known in China as a source of herbal medicine, were also used in former years as a toxin against aphids, mites, and plant-pathogenic fungi. Extracts of *S. chamaejasme* (SC) and seven commercial fungicides were investigated for their inhibition of mycelial growth and germination of cysts of the pathogen *in vitro*. The SC alone, infinito (fluopicolide + propamocab + hydrochloride), fluazinam, dimethomorph, and their mixtures with SC strongly inhibited both mycelial growth and germination of cysts of *P. capsici*. Tests were conducted in a commercial field in Fenggang County, Zunyi City, Guizhou Province, China, in 2019, 2020, 2021, and 2022 to validate these results for growers. A soil application (7 days before transplanting) and three foliar sprays at 7, 10, or 15 days were tested. One soil application of SC followed by three successive foliar sprays of infinito, fluazinam, and dimethomorph at 7- or 10-day intervals, as well as tank-mixed applications of these synthetic fungicides with SC at 15-day intervals, limited Phytophthora blight incidence to < 10% on the non-treated control treatment. Disease incidence was limited to < 3% to < 5% when alternating SC, SC + infinito, SC + fluazinam, and SC + dimethomorph three times at 7-, 10- or 15-day intervals, plus one soil application of SC. When treatments were applied from early April to May, disease incidence was < 10% in the non-treated control during June and July. The optimal concentration of SC was determined to be 0.25 g/L, which effectively controlled Phytophthora blight and protected marketable yield. SC also significantly ($P < 0.05$) outperformed treatments in a nearby commercial pepper field that relied on applications of a single synthetic chemical fungicide. The findings of this study provide a foundation for guiding growers to implement an efficient and environmentally safe spray program against Phytophthora blight of pepper in Guizhou Province.

Keywords: pepper blight; *Phytophthora capsici*; *Capsicum annuum;* biological control; commercial fungicides

Pepper (*Capsicum annuum*), a native of Central and South America, is the third largest vegetable crop in the world after beans and tomatoes. The global pepper planting area is about 1.99 million ha (FAO, 2020). In China, planting area and output accounted for 39.3% and 54.2% of the world total, respectively, mainly distributed in Guizhou, Henan, Yunnan, Sichuan, Xinjiang, Gansu, and Shaanxi provinces.

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Phytophthora blight, caused by the oomycete pathogen *Phytophthora capsici* Leonian, is a destructive disease in pepper production. Since it was discovered in New Mexico in 1918 (Leonian 1922), it has become a worldwide problem (Zhou et al. 1981; Tang et al. 1990; Pennisi et al. 1998). Symptoms include withered leaves, rotten fruit, necrotic spots on stems, wilting, and death. The pathogen spreads through seeds, diseased plants, and by movement of soil and rain. When conditions are highly favourable for the disease, outbreaks can expand so rapidly that they are challenging to control (Aguirreole et al. 1995). In Guizhou province, the largest pepper-producing province for field planting (Lu et al. 2021), Phytophthora blight is particularly serious because of continuous cropping and persistent high temperatures and humidity. Disease incidence (exhibiting leaf or stem symptoms and defoliation) ranges from 30% to 70% and can reach 90% in some fields. (Yang et al. 2002; Li et al. 2007; Chen et al. 2015).

Breeding disease-resistant varieties and pre-seed soil treatment are important strategies to forestall outbreaks (Alcazar et al. 1995; Babadoost et al. 2003). However, the scarcity of commercial cultivars with resistance to this disease and the high cost of chemical seed treatments limit the application of these approaches (Yi et al. 2002). Consequently, spray application of fungicides with activity against the pathogen remains the predominant control method in China. However, long-term exclusive reliance on fungicides, coupled with the pathogen's adaptability to the single-site mode of action fungicides, is gradually undermining the efficacy of this approach (He 2014). Dimethomorph, fluazinam, and kresoxim-methyl are the most widely used and effective fungicides against Phytophthora blight in Guizhou province (Dong et al. 2012; Chen et al. 2014), but all are at risk for resistance development.

The application of extracts from several plant taxa can expand the range of options for plant disease control, as well as reduce reliance on synthetic chemical pesticides and lessen the risk of developing resistance to these products (Wang & Liu 1996; Mendk et al. 2001; Cao et al. 2012; Rongai et al. 2016; Yeole et al. 2016).

The anti-fungal activity of plant extracts was widely used to control *Phytophthora* disease. Extracts from *Stellera chamaejasma* could effectively inhibit *P. infestans* (Min et al. 2007). Citronella (*Cymbopogon nardus*) and garlic (*Allium sativum* L.) extracts showed effectiveness in inhibiting *P. infestans* (Mont.) de Bary on potatoes (Cao & Bruggen 2001).

S. chamaejasme Linn., an herbaceous perennial plant, was first recorded in Shennong's Herbal Classic in 1590 as a Chinese herbal medicine (Feng & Tetsuro 1995). Medicine from the roots of this plant can be used to eliminate phlegm, resolve constipation, and relieve pain (Feng & Tetsuro 1995; Shi 1997). *S. chamaejasme* plants are common in the northern and southwestern provinces of China.

The extraction and pharmacological effects of *S. chamaejasme* have been studied extensively. In a test of the effectiveness of *S. chamaejasme* root extracts against the insect pests *Ostrinia furnacalis* Guenee, *Pierisrapae* Linnaeus (Tan 2002), *Myzus persicae* Sulzer (Xiang et al. 2001), and *Tetranychus viennensis* Zacher (Zhang et al. 2000) larval death rates of > 90% were reported (Lv et al. 2004). The results of *in vitro* tests showed significant anti-fungal activity of *S. chamaejasme* root extract against the phytopathogens *P. infestans*, *Alternaria mali*, and *Fusarium* sp. (Min et al. 2007; Zhao et al. 2010; Cao et al. 2012; Wei et al. 2021).

The present research assessed the activity of *S. chamaejasme* extracts against *P. capsici*. Field tests of these extracts, alone and in combination with synthetic fungicide applications for Phytophthora blight suppression, evaluated the potential of these extracts to provide a practical alternative or supplement to chemical fungicides for control of this devastating disease of commercial pepper production in rural areas of China.

MATERIAL AND METHODS

Plant materials

Mature plants of *S. chamaejasme* were collected from Duolun Nuoer town, Duolun county, Inner Mongolia Autonomous Region, on August 20, 2018. The mixed material, including shoots and roots, was cleaned and dried for 72 h at 40 °C. All dried materials were ground with a small medical pulveriser (Model: YK-11, Yikang Chinese Medicine Mechanism Parts Factory, Qingzhou, Shandong, China), sieved by a 40-mesh sieve (aperture 0.37 mm), and stored in a refrigerator at 0 to 4 °C.

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Extraction of antimicrobial components from *S. chamaejasme*

The Soxhlet method (Matusiewicz, 1982) was used to prepare plant extracts. After 50 g samples of crushed and sieved material were placed in a filter paper cylinder, extraction was performed in a Soxhlet apparatus for 24 h with methanol as the solvent. A rotary evaporator concentrated the extracted solution of each sample and then transferred it to a 50 mL volumetric flask. Neutral alumina (activated at 600 °C for six hours) and silica gel (activated at 110 °C for two hours) were packed into chromatography columns at a ratio of 1 : 3. The separated extracts of *S. chamaejasme* that passed through the column were termed SC.

Oomycete pathogen

An isolate of *Phytophthora capsici* (GZ1654) was obtained from the Fungal Herbarium of Northwest A&F University (HMUABO), Yangling, Shaanxi, China. To assess mycelial growth and zoospore germination of the isolate before use for *in vitro* experiments, the isolate was cultured on oatmeal agar (OA; 30 g of oat powder, model: M523-01, Dingzhou Bioseth Biology Science and Technology Co., Ltd; 50 mL of water) at 25 °C in darkness for one month.

Screening sensitivity to fungicides *in vitro*

Seven commercial fungicides – chlorothalonil, metalaxyl, mandipropamid, infinito (fluopicolide + propamocab + hydrochloride), dimethomorph, kresoxim-methyl, and fluazinam, as well as suspension concentrate (SC) were used to assess *in vitro* suppression of *P. capsici* mycelial growth and zoospore germination (Table 1). Five concentrations of each test material (1 000, 500, 250, 125, and 62.5 μg/mL for SC, kresoxim-methyl, and fluazinam; 800, 400, 200, 100, and 50 μg/mL for chlorothalonil, infinito, and dimethomorph; 600, 300, 150, 75, and 37.5 μg/mL for metalaxyl and mandipropamid), were added to 2% water agar. All concentrations were determined according to the manufacturer's recommended concentration for each fungicide. Distilled water added to 2% WA was used as the control.

The isolate of *P. capsici* (GZ1654) was transferred to oatmeal agar and incubated in darkness at 28 °C for five days, then moved into a well-lit box (4 000 lx, 12 hours/day) for seven days to induce the formation of sporangia. After sporangia appeared, 10 mL of sterile water was added to the petri dish, which was then placed at 4 °C for one hour, followed by 25 °C for one hour to produce zoospores. Zoospores were dislodged with a sterile brush, after which the liquid was filtered with Miracloth (polyester synthetic fibre filter cloth, aperture 22–25 μm; Calbiochem, Merck Co. Inc., Darmstadt, Germany) to remove impurities. Zoospore concentration was adjusted to 1–5 × 10^5 zoospores/mL by appropriate

Table 1. Commercial fungicides used for *in vitro* and in vivo testing against *P. capsici* and Phytophthora blight of pepper, respectively

Active ingredient[a]	Trade name[b]	Chemical family	Mode of action[c]	FRAC code[c]	Formulation[d]	Manufacturer
Chlorothalonil (56)	Shelcore	Substituted benzene	unknown	36	EW	Syngenta
Metalaxyl (25)	Aplon	Acylamide	A1: RNA polymmerase I	4	WG	Bayer
Mandipropamid (23.4)	Ruifan	Acylamide	A1: RNA polymmerase I	4	SC	Syngenta
Infinito (fluopicolide+propamocab+hydrochloride) (68.75)	Infinito	Pyrazolamide	C2: complex II: succinate-dehydrogenase	7	SC	Bayer
Dimethomorph (50)	Acrobat	Morpholine	G2: Δ14-reductase and Δ^8—›Δ^7- isomerase in sterol biosynthesis (*erg 24*, *erg2*)	5	WG	BASF
Kresoxim-methyl (50)	Green Bay	Methoxyl acrylic ester	C3: complex III: cytochrome bc 1 (ubiquinol oxidase) at Qo site (*cyt b gene*)	11	WG	BASF
Fluazinam (50)	Chigon	Dinitroanilines	C5: uncouplers of oxidative phosphorylation	29	SC	NPX

[a]Number in parentheses indicates the percentage of active ingredients in commercial products; [b]products marketed and sold in China and used in this research; [c]FRAC code list©*: fungicides sorted by mode of action (including FRAC code numbering); [d]SC – suspension concentrate; WG – water dispersible granule; EW – emulsion in water

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dilutions with sterile water based on hemacytometer counts (only motile zoospores counted).

To make mycelial culture suspensions, 10 μL of a suspension of 5 × 10^5 zoospores/mL of zoospore were incubated and placed in the center of the plate (Petri dish with a diameter of 9 cm) with oatmeal agar and cultured for a week in darkness at 25 °C. Subsequently, a 0.5 cm diameter agar disk subsampled from the margin of the colony was inoculated into Erlenmeyer flasks containing 50 mL oatmeal medium (OM; 30 g of oat powder, model: WF1350, Dingzhou Bioseth Biology Science and Technology Co., Ltd; 50 mL of water) and mixed with five different concentrations of fungicides and SC extract. Three replicate flasks were prepared per treatment, and OM alone was used as the control. After incubation on a shaker table (100 rpm) at 25 °C for 14 days, mycelium was filtered through a Buchner funnel with filter paper (110 mm, Fushun Dongyang Industrial and Trading Co. Ltd, Fushun, China) and weighed after drying for one hour at 80 °C. Growth inhibition percentage was calculated as [(mycelium dry weight of control) – (mycelium dry weight of treatment)] / (mycelium dry weight of control) × 100.

10 μL of a 5 × 105 zoospores/mL suspension were transferred with a pipet into plastic Petri dishes (94 × 16 mm) containing 2% WA medium and spread evenly using a sterile glass rod. Three replicate plates were prepared per treatment. All cysts and encysted cysts on the plates were counted using a microscope (Olympus-CX23) after incubation for 12 h in darkness at 25 °C with relative humidity > 90%. Germination inhibition percentage was calculated as [(number of cysts in nonfungicide control) – (number of cysts in treatment)] / (number of cysts in nonfungicide control) × 100.

Location and plant material for field trials

Field studies were conducted in 2019, 2020, 2021, and 2022 in a commercial pepper field in Fuchuan town, Fenggang County, Zunyi City, Guizhou Province (107°39'E, 27°41'N). Hothouse-grown, one-month-old seedlings of hot pepper (cv. Zun No.1) were transplanted in rows on raised beds at a spacing of 30 cm within rows and 45 cm between rows on March 31, 2019, April 10, 2020, April 10, 2021, and April 5, 2022. Nonsprayed guard rows separated treatment rows. Zoospores of *P. capsici* (GZ1654) were used for inoculation after appropriate dilutions with sterile water. Pepper plants (leaves and stems) were inoculated seven days after transplanting on April 7, 2019, April 17, 2020, April 17, 2021, and April 12, 2022. 20 mL of zoospores, a suspension of 5 × 10^5 zoospores/mL, were sprayed on each plant by a handheld sprinkling can (the volume was 500 mL, DL581005, Deli Group Limited).

The experimental design was a randomized block experiment incorporating 50 contiguous plants per subplot and four replicate plots per treatment. The different field in the same town was used in all four years of the study, and subplot locations were rerandomized each year.

2019 and 2020 trials. The most effective fungicides and concentrations from *in vitro* trials were selected for the field trials. Three systemic fungicides and SC were tested to compare their

Table 2. Fungicide treatments for control of Phytophthora blight of pepper in Fuchuan town, Fenggang County, Zunyi city, Guizhou Province, during 2019–2022

Treatment	Sequence of fungicide applications			
	Soil application	Foliar sprays		
	first application	second application	third application	fourth application
1	SC	infinito	infinito	infinito
2	SC	fluazinam	fluazinam	fluazinam
3	SC	dimethomorph	dimethomorph	dimethomorph
4	SC	SC	SC	SC
5	SC	SC + infinito	SC + infinito	SC + infinito
6	SC	SC + fluazinam	SC + fluazinam	SC + fluazinam
7	SC	SC + dimethomorph	SC + dimethomorph	SC + dimethomorph

Materials were diluted in water to the following concentrations of active ingredient: infinito (0.25 g/L), fluazinam (0.4 g/L), dimethomorph (0.15 g/L), and SC (0.25 g/L), sprays were applied at the rate of 3 000 L per hectare; all sprays were applied to runoff with a backpack, hand-powered sprayer with a hollow cone nozzle (1.2 mm diameter spray aperture)

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impact on the incidence of symptoms caused by *P. capsici*. Nine treatments (spray-applied single materials or tank-mixed combinations at different intervals three or four times per season (Table 2) included: *(i)* treatments evaluating single anti-fungal products SC, infinito, fluazinam, and dimethomorph, respectively, *(ii)* successive treatments of SC + infinito, SC + fluazinam, and SC + dimethomorph, respectively. The control treatment received no sprays.

In each year, intervals between applications included 7, 10 days, or 15 days (a total of four sprays per growing season). In all treatments except the control, the initial spray was applied to the soil surface seven days before transplanting (March 24, 2019 and April 3, 2020). The time interval between the initial application and transplanting was consistent with intervals between applications, and all second applications were made on the same day five days after inoculation (March 12, 2019, and April 22, 2020), regardless of the spray interval. The volumes applied for soil treatment were the same as for foliar sprays (Table 2). Two surveys of disease incidence (percentage of plants exhibiting leaf or stem symptoms) at 10 (May 22, 2019, and June 2, 2020) and 40 days (June 22, 2019 and July 2, 2020) after the last sprays were implemented to assess symptom development of Phytophthora blight after anti-fungal sprays (Table 3). All treatments for a given application interval were applied on the same day.

These treatments also included a comparison of single-fungicide with tank mixes of SC plus other fungicides, and different time intervals between sprays (7, 10, or 15 days) in 2019 and 2020 (Tables 2 and 3).

2021 and 2022 trials. Disease progress after spraying was also monitored in 2021 and 2022. The first application, spraying the treatments on the soil surface, was made on April 3, 2021, and March 29, 2022. Subsequently, three independent treatments were evaluated: *(i)* successive treatments of SC + infinito, SC + fluazinam, and SC + dimethomorph; *(ii)* alternating applications of SC, infinito, fluazinam, and dimethomorph; and *(iii)* alternating applications of SC, SC + infinito, SC + fluazinam, and SC + dimethomorph. These treatments also encompassed at intervals of 7 (April 22, April 29, and May 6, 2021; April 17, April 24, and May 1, 2022), 10 (April 22, May 2, and May 12, 2021; April 17, April 27, and May 7, 2022), and 15 days (April 22, May 7, and May 22, 2021; April 17, May 2, and May 17, 2022). Disease incidence was surveyed at 10 (June 2, 2021 and May 27, 2022) and 40 (July 2, 2021 and June 27, 2022) days after the last spray (Table 3). The non-sprayed treatment was used as a control.

Based on the results of the 2019 and 2020 field trials, an additional field trial was designed in 2021 and 2022. Treatments included alternating applications of SC, SC + infinito, SC + fluazinam, and SC + dimethomorph, with different concentrations of SC (3, 2, 1, 0.5, 0.25, and 0.125 g/L) sprayed at 10-day intervals, respectively, during the season. Disease incidence, yield (fruits from all 50 plants per plot harvested), height (measured from the soil surface to the top of the plant with a soft ruler), and stem diameter (the diameter of the main stem at 10 cm from the soil surface was measured with a soft ruler) of the plants were measured at harvest on August 1, 2021, and August 10, 2022.

All sprays were applied to runoff with a backpack, hand-powered sprayer with a hollow cone nozzle (1.2 mm diameter spray aperture).

Table 3. Date of sprays and surveys for control of Phytophthora blight of pepper in Fuchuan town, Fenggang County, Zunyi City, Guizhou Province, during 2019–2022

Sprays date			Surveys date
7-day intervals	10-day intervals	15-day intervals	
2019			
March 24, April 12, April 19, April 26	March 24, April 12, April 22, May 2	March 24, April 12, April 27, May 12	May 22, June 22
2020			
April 3, April 22, April 29, May 3	April 22, May 2, May 12	April 3, April 22, May 7, May 22	June 2, July 2
2021			
April 3, April 22, April 29, May 6	April 3, April 22, May 2, May 12	April 3, April 22, May 7, May 22	June 2, July 2
2022			
April 7, April 24, May 1	April 17, April 27, May 7	April 17, May 2, May 17	May 27, June 27

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Disease evaluation

Fifty plants per subplot were rated individually for the presence of symptoms of Phytophthora blight. Leaf scars were counted as evidence of defoliation, and dead plants were counted among diseased plants.

Disease incidence was expressed as the number of diseased plants (exhibiting leaf or stem symptoms and defoliation) /number of investigated plants × 100.

Plant height, stem diameter, and yield were measured at harvest. These parameters were also measured on the exact dates in nearby commercial pepper fields (the same cultivar as the test fields). These 10 ha fields were 50 m from our experimental field. Five subplots were randomly sampled, and 50 plants were surveyed in each subplot.

Data analysis

To evaluate *in vitro* mycelia growth and germination of cysts data, statistical analyses were performed using linear regression (SAS 9.2, SAS Institute Inc.). Values of median effective concentration for 50% inhibition of mycelial growth and germination of cysts (EC50) were calculated. Homogeneity of error variance was tested by using PROC GLM of SAS, and *in vitro* inhibition; data were pooled when homogeneity criteria were met. Mean EC50 values were compared using Tukey's honestly significant difference (HSD) test at $\alpha = 0.05$. Treatment means calculated in the field experiments were compared with each other on each sampling date using two-way ANOVA in PROC GLM of SAS. Data for each year of fungicide trials were analyzed separately by ANOVA for a split-plot design using Mixed Model Proceed in SAS. The individual test year data were analyzed separately based on significant test effects in preliminary analyses. Significant differences among spray-interval treatments (7, 10, or 15 days) within a rating category (either incidence of plant symptoms or growth of pepper plants) and fungicide regime within a year were determined using Tukey's HSD mean separation test ($P = 0.05$). The effects of all treatments (fungicide, spray intervals, and concentration) and plots were included in the model. The fungicide, spray intervals, and concentration were treated as fixed effects, whereas the plot and subplot were treated as random effects.

RESULTS

***In vitro* sensitivity**

Infinito, dimethomorph, fluazinam, and SC exhibited strong inhibition of both mycelial growth and germination of cysts of *P. capsici* compared with the control (Table 4). The inhibition ability of kresoxim-methyl was strong to mycelial growth but weak to germination of cysts. Chlorothalonil, metalaxyl, and mandipropamid inhibited mycelial growth and germination of cysts with intermediate effectiveness (Table 4).

Field experiments

2019 and 2020 trials. Infinito, fluazinam, and dimethomorph provided the most effective control of Phytophthora blight of pepper among the single-fungicide treatments applied every seven days, with disease incidence < 10% in 2019 and 2020. (Table 5). The SC was slightly less effective; disease incidence was 11.8% in 2019 and 7.6% in 2020 (Table 5).

Table 4. Inhibitory effect of fungicides against *P. capsici in vitro*

Fungicides	Mean EC_{50} (μg/mL)*	
	Mycelial growth	Germination of cysts
Control	315.2 ± 5.1^a	372.8 ± 3.0^a
Chlorothalonil	177.9 ± 1.5^b	208.2 ± 1.0^b
Mandipropamid	185.1 ± 0.8^b	146.2 ± 0.9^c
Metalaxyl	113.2 ± 0.9^c	138.9 ± 1.0^c
Kresoxim-methyl	95.7 ± 1.3^d	215.3 ± 2.4^b
Dimethomorph	37.6 ± 0.1^e	25.3 ± 0.9^d
Infinito	15.5 ± 0.2^f	21.2 ± 0.3^d
Fluazinam	20.4 ± 0.6^f	29.2 ± 0.7^d
P-Value	< 0.001	< 0.001

*Effective concentration to inhibit 50% growth ± standard deviation (n =3); mycelia were harvested on an Oatmeal medium with different fungicides, the zoospore germination rate of *P. capsici* isolate GZ1654 was examined after 12 h of incubation at 25 °C in darkness on 2% WA plates that were amended with different fungicides; oatmeal medium without fungicide as the control; values followed by the same letters are not significantly ($P < 0.05$) different based on Tukey's honest significant difference (HSD) mean separation test

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Tank-mixed applications of infinito, fluazinam, or dimethomorph with SC were significantly ($P < 0.05$) more effective than infinito, fluazinam, or dimethomorph alone, reducing disease incidence to < 5% (Table 5).

One soil application of SC and three successive foliar sprays of infinito, fluazinam, and dimethomorph alone at 7- or 10-day intervals suppressed disease incidence to < 10% or < 15%, respectively. However, the incidence was > 17% when the application was sprayed at 15-day intervals (Table 5).

One soil application of SC and three successive foliar applications of SC + infinito, SC + fluazinam, and SC + dimethomorph three times at 7- or 10-

Table 5. Control of Phytophthora blight by a range of application schedules of anti-fungal materials on pepper in a field in Fuchuan town, Fenggang County, Zunyi City, Guizhou Province, during 2019 and 2020

Treatments[a]	Disease incidence (% symptomatic plants) during the first survey[b]					
	7-day intervals		10-day intervals		15-day intervals	
	2019	2020	2019	2020	2019	2020
Treatment 1	9.5 ± 1.2[c]	6.3 ± 1.2[d]	14.8 ± 0.7[c]	10.9 ± 1.4[c]	22.3 ± 1.0[c]	17.4 ± 0.9[c]
Treatment 2	9.7 ± 0.4[c]	6.1 ± 0.8[d]	15.0 ± 0.8[c]	11.0 ± 0.9[c]	21.9 ± 0.3[c]	17.0 ± 0.8[c]
Treatment 3	9.9 ± 0.9[c]	7.3 ± 0.8[c]	14.3 ± 1.1[c]	11.4 ± 0.5[c]	23.6 ± 0.7[c]	17.4 ± 1.1[c]
Treatment 4	11.8 ± 0.6[b]	7.6 ± 1.1[b]	17.2 ± 0.3[b]	13.9 ± 0.7[b]	29.5 ± 0.6[b]	21.1 ± 0.9[b]
Treatment 5	4.0 ± 0.8[e]	2.3 ± 0.5[f]	6.0 ± 0.4[e]	3.0 ± 0.9[e]	9.9 ± 0.7[e]	6.2 ± 0.6[e]
Treatment 6	4.8 ± 0.5[e]	2.1 ± 0.8[f]	7.0 ± 1.2[d]	4.8 ± 0.8[de]	9.7 ± 0.8[e]	6.9 ± 1.0[e]
Treatment 7	4.5 ± 0.8[e]	2.4 ± 0.3[f]	7.4 ± 0.8[d]	5.6 ± 0.7[d]	9.9 ± 0.7[e]	6.5 ± 0.7[e]
Control	58.6 ± 0.9[a]	33.9 ± 0.5[a]	58.6 ± 0.9[a]	33.9 ± 0.5[a]	58.6 ± 0.9[a]	33.9 ± 0.5[a]
F-value	30.3	29.3	27.5	27.1	30.5	19.8
P-value	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Treatments[a]	Disease incidence (% symptomatic plants) during the second survey					
	7-day intervals		10-day intervals		15-day intervals	
	2019	2020	2019	2020	2019	2020
Treatment 1	38.3 ± 1.7[c]	28.5 ± 1.1[c]	44.7 ± 0.9[c]	23.5 ± 1.5[c]	51.3 ± 1.3[c]	35.7 ± 1.0[c]
Treatment 2	38.5 ± 0.9[c]	29.1 ± 1.7[c]	44.3 ± 1.4[c]	24.1 ± 1.9[c]	52.1 ± 1.2[c]	36.8 ± 1.7[c]
Treatment 3	39.2 ± 1.1[c]	29.7 ± 0.9[c]	24.1 ± 1.9[c]	23.9 ± 0.8[c]	51.7 ± 2.7[c]	35.5 ± 2.5[c]
Treatment 4	42.8 ± 0.8[b]	30.9 ± 1.3[b]	48.3 ± 0.8[b]	29.4 ± 1.3[b]	57.9 ± 2.2[b]	41.0 ± 1.3[b]
Treatment 5	25.7 ± 0.9[e]	12.9 ± 0.7[e]	26.8 ± 1.5[e]	15.3 ± 1.0[e]	41.3 ± 1.8[e]	21.7 ± 1.3[e]
Treatment 6	26.2 ± 0.7[e]	14.1 ± 0.8[e]	27.5 ± 0.7[e]	16.4 ± 0.9[e]	41.1 ± 1.2[e]	21.3 ± 1.6[e]
Treatment 7	26.9 ± 1.3[e]	13.5 ± 0.9[e]	27.8 ± 1.0[e]	16.7 ± 0.9[e]	40.9 ± 1.5[e]	22.0 ± 0.9[e]
Control	88.3 ± 1.7[a]	65.3 ± 0.9[a]	88.3 ± 1.7[a]	65.3 ± 0.9[a]	88.3 ± 1.7[a]	65.3 ± 0.9[a]
F-value	27.3	21.1	22.2	25.7	23.9	21.1
P-value	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01

[a]Treatment 1 — successive applications of infinito alone; Treatment 2 — successive applications of fluazinam alone; Treatment 3 — successive applications of dimethomorph alone; Treatment 4 — successive applications of SC alone; Treatment 5 — successive applications of SC + infinito; Treatment 6 — successive applications of SC + fluazinam; Treatment 7 — successive applications of SC + dimethomorph

[b]Treatments were applied either four times at 7-, 10- or 15-day intervals. The initial spray was applied to the soil surface before transplanting. In 2019 trials, treatments were sprayed on March 24, April 12, April 19, and April 26 (at 7-day intervals), March 24, April 12, April 22, and May 2 (at 10-day intervals), March 24, April 12, April 27, and May 12 (at 15-day intervals). In 2020 trials, treatments were sprayed at 7-day (April 3, April 22, April 29, and May 6), 10-day (April 3, April 22, May 2, and May 12); or 15-day (April 3, April 22, May 7, and May 22) intervals.

Disease incidence was estimated on May 22, and June 22, 2019, and June 2, and July 2, 2020. $n = 4$

Treatment means showing the same letters are not significantly ($P < 0.05$) different based on Tukey's honest significant difference (HSD) mean separation test

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day intervals after soil application of SC suppressed the disease incidence to < 5% and 10%, respectively. Three tank-mixed applications at intervals of 15 days, plus a soil application of SC, also limited disease incidence to < 10% (Table 5).

2021 and 2022 trials. Following an initial soil application of SC, three successive foliar sprays of SC + infinito, SC + fluazinam, and SC + dimethomorph at 7-, 10-, and 15-day intervals suppressed symptoms to < 10% in 2021 and 2022. However, incidence rose to > 30% (7-day intervals) and > 40% (10 or 15-day intervals) 40 days after the last application (Table 6).

Alternating applications of SC, infinito, fluazinam, and dimethomorph at intervals of 7 and 10 days limited disease incidence to < 10% and < 15% at intervals of 15 days in 2021 and 2022 but to > 35% forty days after the last application (Table 6).

Alternating applications of SC, SC + infinito, SC + fluazinam, and SC + dimethomorph provided consistent control (disease incidence was < 5% in 2021 and 2022) when alternated at 7, 10, or 15 days intervals. At 40 days since the last application, incidence was still < 10% (Table 6).

Alternating applications of SC, SC + infinito, SC + fluazinam, and SC + dimethomorph under differ-

Table 6. Consistent control effect of anti-fungal sprays on Phytophthora blight of pepper during 2021 and 2022 in a field in Fuchuan town, Fenggang County, Zunyi City, Guizhou Province

Treatments[a]	Disease incidence (% symptomatic plants) during the first survey[b]					
	7-day intervals		10-day intervals		15-day intervals	
	2019	2020	2019	2020	2019	2020
Treatment 1	4.7 ± 0.3^{c}	3.1 ± 0.7^{c}	6.4 ± 1.2^{b}	3.2 ± 1.2^{c}	8.4 ± 1.0^{c}	5.3 ± 1.1^{c}
Treatment 2	4.8 ± 0.6^{c}	3.3 ± 0.6^{c}	6.9 ± 0.9^{b}	3.8 ± 1.1^{c}	8.7 ± 0.7^{c}	5.9 ± 1.0^{c}
Treatment 3	4.8 ± 1.0^{c}	3.4 ± 0.6^{c}	7.1 ± 1.3^{b}	3.6 ± 0.9^{c}	8.7 ± 0.5^{c}	5.8 ± 1.0^{c}
Treatment 4	7.1 ± 0.5^{b}	5.3 ± 0.4^{b}	9.8 ± 0.7^{b}	5.9 ± 0.3^{b}	14.9 ± 1.3^{b}	10.3 ± 1.3^{b}
Treatment 5	3.5 ± 0.9^{d}	1.1 ± 0.5^{d}	3.8 ± 0.8^{d}	1.9 ± 0.8^{d}	4.3 ± 0.5^{d}	3.8 ± 0.4^{d}
Control	45.2 ± 0.4^{a}	27.6 ± 1.0^{a}	45.2 ± 0.4^{a}	27.6 ± 1.0^{a}	45.2 ± 0.4^{a}	27.6 ± 1.0^{a}
F-value	32.5	22.4	34.6	25.4	33.4	27.5
P-value	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Treatments[a]	Disease incidence (% symptomatic plants) during the second survey[b]					
	7-day intervals		10-day intervals		15-day intervals	
	2021	2022	2021	2022	2021	2022
Treatment 1	30.1 ± 1.3^{c}	21.4 ± 0.9^{c}	40.3 ± 1.6^{c}	28.6 ± 0.9^{c}	45.0 ± 1.1^{c}	32.8 ± 1.2^{c}
Treatment 2	31.2 ± 1.5^{c}	20.3 ± 0.8^{c}	41.3 ± 1.2^{c}	28.1 ± 0.7^{c}	44.8 ± 1.2^{c}	33.3 ± 0.8^{c}
Treatment 3	30.9 ± 0.9^{c}	20.9 ± 1.8^{c}	40.1 ± 1.9^{c}	29.8 ± 1.0^{c}	46.1 ± 1.4^{c}	33.5 ± 1.6^{c}
Treatment 4	37.1 ± 0.9^{b}	28.1 ± 1.0^{b}	47.1 ± 0.8^{b}	35.3 ± 0.9^{b}	54.1 ± 0.7^{b}	41.7 ± 1.0^{b}
Treatment 5	7.4 ± 1.2^{d}	5.7 ± 0.8^{d}	8.2 ± 0.3^{d}	6.5 ± 0.6^{d}	8.9 ± 0.5^{d}	7.3 ± 1.0^{d}
Control	73.9 ± 1.4^{a}	53.5 ± 0.5^{a}	73.9 ± 1.4^{a}	53.5 ± 0.5^{a}	73.9 ± 1.4^{a}	53.5 ± 0.5^{a}
F-value	29.7	35.4	28.9	32.4	25.0	25.7
P-value	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01

[a]Treatment 1 — successive applications of SC + infinito; Treatment 2 — successive applications of SC + fluazinam; Treatment 3 — successive applications of SC + dimethomorph; Treatment 4 — alternating applications of SC, infinito, fluazinam, and dimethomorph; Treatment 5 — alternating applications of SC, SC + infinito, SC + fluazinam, and SC + dimethomorph
[b]Treatments were applied either four times at 7-, 10- or 15-day intervals. The initial spray was applied to the soil surface before transplanting. In 2021 trials, treatment was sprayed on April 3, April 22, April 29, and May 6 (at 7-day intervals), April 3, April 22, May 2, and May 12 (at 10-day intervals), April 3, April 22, May 7, and May 22 (at 15-day intervals). In 2022 trials, treatments were sprayed on 7 days (April 17, April 24, and May 1), 10 days (April 17, April 27, and May 7), and 15 days (April 17, May 2, and May 17)
Disease incidence was estimated on June 2, July 2, 2021, May 27, and June 27, 2022. $n = 4$; treatment means showing the same letters are not significantly ($P < 0.05$) different based on Tukey's honest significant difference (HSD) mean separation test

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Table 7. Impact of concentrations of SC on the incidence of Phytophthora blight and growth increment of pepper during 2021 and 2022 in a field in Fuchuan town, Fenggang County, Zunyi city, Guizhou Province

Treatments[a]	Plant height (cm)		Stem diameter (mm)		Yield (t/ha)		Disease incidence (% symptomatic plants)	
	2021	2022	2021	2022	2021	2022	2021	2022
3 g/L	92.2 ± 1.5[a]	93.8 ± 1.4[a]	93.2 ± 1.4[a]	94.6 ± 0.9[a]	32.3 ± 0.7[a]	31.2 ± 0.6[a]	4.3 ± 0.9[d]	2.0 ± 0.9[d]
2 g/L	89.1 ± 1.7[b]	89.8 ± 0.9[b]	90.6 ± 1.3[b]	90.0 ± 1.5[b]	29.2 ± 0.6[b]	28.2 ± 0.4[b]	5.0 ± 0.9[cd]	2.9 ± 0.8[cd]
1 g/L	89.0 ± 1.4[b]	88.3 ± 1.5[b]	91.0 ± 1.6[b]	88.7 ± 1.6[b]	28.0 ± 1.0[b]	27.9 ± 0.9[b]	5.6 ± 0.6[cd]	6.9 ± 0.4[c]
0.5 g/L	88.3 ± 1.9[b]	87.0 ± 1.0[b]	89.3 ± 1.6[b]	88.2 ± 0.9[b]	27.9 ± 0.7[b]	27.5 ± 0.6[b]	6.9 ± 0.7[c]	4.6 ± 0.9[c]
0.25 g/L	87.3 ± 1.9[b]	86.1 ± 0.9[b]	89.3 ± 0.8[b]	87.1 ± 0.8[b]	27.9 ± 0.9[b]	26.0 ± 0.4[b]	7.2 ± 0.3[c]	6.5 ± 0.6[c]
0.125 g/L	77.3 ± 2.0[c]	78.3 ± 1.3[c]	79.7 ± 0.9[c]	81.4 ± 0.7[c]	20.6 ± 0.5[c]	23.1 ± 0.7[c]	14.5 ± 1.2[b]	8.9 ± 0.9[b]
Control	44.5 ± 1.5[d]	41.3 ± 1.4[d]	60.9 ± 1.5[d]	59.5 ± 1.1[d]	7.0 ± 1.0[d]	8.9 ± 1.5[d]	81.9 ± 1.5[a]	68.5 ± 0.9[a]
F-value	17.4	23.6	15.7	19.3	31.3	25.4	19.9	17.6
P-value	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01

[a]Alternating applications of SC, SC + infinito, SC + fluazinam, and SC + dimethomorph, with different concentrations of SC (3, 2, 1, 0.5, 0.25, and 0.125 g/L) sprayed at 10-day intervals (April 10, April 20, and April 30, 2021; April 5, April 15, and April 25, 2022) The disease incidence, the height and stem diameter of the plants, and yield were measured on August 1, 2021, and August 10, 2022, $n = 4$; treatment means showing the same letters are not significantly ($P < 0.05$) different based on Tukey's honest significant difference (HSD) mean separation test

ent concentrations of SC sprayed at 10-day intervals in 2021 and 2022 resulted in incidence of Phytophthora blight of pepper that was significantly ($P < 0.05$) lower than the control. Disease incidence was < 10% under SC concentrations of 3, 2, 1, 0.5, and 0.25 g/L and < 15% at 0.125 g/L (Table 7).

DISCUSSION

We provide the first evidence that *S. chamaejasme* extracts (SC) can supplement or even replace applications of synthetic chemical fungicides for control of Phytophthora blight of pepper, offering a potentially more sustainable and less hazardous approach for this economically damaging disease in outdoor production fields.

The *S. chamaejasme* plant has long been used for control of insect pests and injurious microbes (Zhang et al. 2000). Extracts of this species have potential for development as a commercial botanical fungicide product (Zhao et al. 2010) given the liabilities of cost, health risks, and negative environmental impact associated with synthetic chemical fungicides. Our findings add evidence in support of that assumption. We tank-mixed or alternated SC with synthetic chemical fungicides and obtained substantial disease suppression from both strategies. *In vitro* trials showed that SC, infinito, fluazinam, and dimethomorph strongly inhibited mycelial growth and encysted zoospore germination of *P. capsici*. Based on field trials in 2019 and 2020, tank mixes of infinito, fluazinam, or dimethomorph with SC limited disease incidence to < 5% and were significantly ($P < 0.05$) more effective in suppressing disease development than infinito, fluazinam, or dimethomorph alone.

Metalaxyl, cymoxanil, propamocarb, oxadixyl, and ethylphosphate were reported to be very effective against *P. capsici* on pepper, but the possible emergence of resistance is in dispute (Li et al. 1995; Pennisi et al. 1998; Luo et al. 1999; Shao et al. 1998; Bi et al. 2002; Kousik & Keinath 2008). Chemical control of plant diseases exerts high selection pressure for developing pathogen resistance to fungicides of *P. infestans* (Lozoya-Saldaa et al. 2017). Agricultural measures and anti-fungal plants were used as complementary and alternative tactics. Extracts from *S. chamaejasma* and others could effectively control potato late blight caused by Phytophthora infestans (Böhm & Cerny 2002; Min et al. 2007; Nyankanga et al. 2008; Nechwatal & Zellner 2013).

Based on our findings, we suggest using infinito, fluazinam, or dimethomorph, which have multiple action sites and no evidence of cross-resistance, and tank-mixing them with SC will minimise the risk of fungicide resistance.

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SC has a strengthening effect as a mixing partner with synthetic fungicides. Applying fungicide sprays at 7-day intervals is a traditional practice for Phytophthora blight of pepper control programs in Guizhou Province (Li et al. 2007). The disease is spread through water, diseased plants, and soil (Li et al. 2007). In the present study, pre-transplant soil application and post-transplant foliar spraying were tested at intervals of 7, 10, or 15 days to suppress *P. capsici* activity. Tank-mixed applications of SC + infinito, SC + fluazinam, and SC + dimethomorph, and alternating application of SC, infinito, fluazinam, and dimethomorph limited incidence to < 5% and to < 10%, and extended the interval to 15 days compared with other trials. These results suggest that alternating fungicide chemistries and widening spray intervals to 15 days could reduce the risk of fungicide resistance.

Pepper can be repeatedly infected by *P. capsici* in the same season, especially during periods of high temperature and humidity (Li et al. 2007). When testing for secondary occurrence of the disease, we found that when alternating applications of SC, SC + infinito, SC + fluazinam, and SC + dimethomorph at intervals of 10 days suppressed the disease to < 3% incidence and the incidence of secondary symptoms incidence was < 10% one month after the final application. There are two peak infection periods of *P. capsici* during late April to early May and late May to mid-July, which is the higher-risk period of the disease epidemic and also is the key period for the growth of pepper in Zunyi City, Guizhou Province (Lai et al. 2016). To slow down the spread of the disease and reduce the disruption of planned spraying by rainfall, we suggest that the first spray should start in early April to suppress early symptoms of Phytophthora blight of pepper to a low level, thereby limiting the amount of inoculum for subsequent dissemination. We recommend alternating application of SC, SC + infinito, SC + fluazinam, and SC + dimethomorph at intervals of 10 days from April to May. Symptoms suppressions in the early infection period limited subsequent disease development, constraining the possibility of outbreaks later in the season. The long-term inhibitory effect was the core element for helping growers avoid late-season sprays to limit residues on harvested fruit and save money.

The epidemic severity of pepper blight is closely related to the marketable yield (Ristaino 1991). In Italy, loss of pepper yield caused by Phytophthora blight reached over 50%, over 70% in Greece, and 40–80% in China (Liang et al. 1992; Ristaino et al. 1992; Ristaino 1993; Ma et al. 1998; Zheng et al. 2006). In our trials, disease incidence was limited to < 10% when alternating SC, SC + infinito, SC + fluazinam, and SC + dimethomorph at 10-day intervals. Correspondingly, the plant height, stem diameter and pepper yield significantly ($P < 0.05$) exceeded observational data from comparison commercial pepper fields. We estimated 0.25 g/L as the optimal SC concentration for commercial pepper fields in Guizhou Province.

These strategies offer potential advantages for growers, providing they can gain affordable access to SC extracts.

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