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Evaluation of Chloropicrin Gelatin Capsule Formulation as a Soil Fumigant for Greenhouse Strawberry in China

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ABSTRACT: Gelatin capsules containing chloropicrin (Pic gel cap) were developed as a new formulation to reduce the potential human exposure risks associated with injection application methods. The objective of this study was to test the efficacy of a Pic gel cap formulation on soilborne pathogens and to determine the effects on strawberry plant growth and fruit yield. Three field experiments were conducted in strawberry greenhouses located in Mancheng County, China, in 2008−2010. The results demonstrated that effects of Pic gel cap on soilborne pathogens were similar to Pic injection; Pic gel cap effectively reduced key soilborne pathogens population, was partially effective against weeds, improved strawberry plant growth, and increased fruit yield significantly compared to the untreated control. Pic gel cap applied to preformed beds uses less fumigant than broadcast applications of Pic gel cap and can provide an equivalent level of disease control. The present study confirms that the Pic gel cap is a promising new formulation which provides field efficacy and marketable yields similar to Pic injection or methyl bromide in strawberry cultivation in China.

KEYWORDS: *chloropicrin, fumigation, gelatin capsule formulation, strawberry, soilborne disease*

INTRODUCTION

The use of methyl bromide (MB) as a preplant soil fumigant for controlling soilborne pests will be phased out in China by 2015. Chloropicrin (Pic) is a potential MB substitute in China, due to its broad biocidal and fungicidal properties and absence of ozone-depleting characteristics. Pic has been used as a soil fumigant in many countries, primarily for high-value crops such as strawberries,[1] peppers, tobacco,[2] flowers,[3] tomato,[4,5] and many others.[6] Pic by itself is as effective as MB for the control of fungal pathogens[7] and improves plant growth and yield responses.[4,8] Of the currently available soil fumigants, Pic is the most efficacious against plant pathogenic fungi.[9,10] Pic was first used for strawberry culture in California to control *Verticillium* wilt.[11] During the past 50 years, intensive strawberry production in most regions worldwide used preplant soil disinfestation with MB and Pic mixtures to control key soilborne pathogens, weeds, and pests.[12]

Mancheng County in Hebei Province cultivates strawberry on about 6000 ha annually and is one of the largest strawberry-producing regions of China. This county has a long history of strawberry production, and soilborne diseases have become a major problem due to continuous cropping. When strawberry is cultivated continuously, the yield generally falls to 60−70% of the normal level. Preplant soil fumigation with Pic is widely used for controlling soilborne pathogens in Mancheng, China.[13] Plant vigor and yields obtained with Pic are similar to those achieved with MB, and these benefits have helped farmers to accept Pic quickly in Mancheng.[14] However, Pic is a strong eye irritant and has a pungent unpleasant smell, which can pose barriers to its adoption. Whereas well-equipped commercial companies generally provide safe soil fumigation services in the United States, Europe, or Japan, soil fumigation in China is mostly conducted by individual farmers who lack essential application tools and personal protection equipment. The gelatin capsule (gel cap) formulation of Pic[15] offers a promising solution to these constraints because it can reduce the risk to workers and bystander exposure during application.

The objective of this study was to test the efficacy of Pic gel cap treatments for the control of key soilborne pathogens (*Fusarium oxysporum* and *Phytophthora* spp.) and to determine the effect of Pic gel cap on strawberry plant growth and marketable yield. Two different application methods of gel cap were evaluated: bed and broadcast fumigation.

MATERIALS AND METHODS

Field Sites. During 2008−2010, three field experiments were conducted in three strawberry greenhouses located in Duanwang village in Mancheng County, Hebei Province. Table 1 summarizes the basic physical and chemical properties of soil at the experimental sites. All of the field sites were located in intensive strawberry production areas of Mancheng. These sites have a long history of strawberry cultivation and suffer substantial pressure from soilborne diseases due to continuous cropping. The major soilborne diseases are caused by *F. oxysporum* and *Phytophthora* spp. Root knot nematodes have not been observed locally.

Experimental Design. Table 2 provides a list of the treatments tested. Each plot area was arranged using a randomized block design, and each treatment was repeated three times. Technical grade Pic (99.5% purity) was purchased from Dalian Dyechem Co. Ltd. (Dalian, China). The gelatin capsules were produced on a common capsule machine that was modified by adding sealing equipment to eliminate Pic emissions during gel cap production. The capsule skin (0.6 mm thickness) was composed of gelatin supplied by Qinghai Gelatin Co. Ltd. On average, each gel cap had a volume of 1 mL and contained 1.13 g of Pic. The gel caps were applied to field soil by forming holes

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Table 1. Physical and Chemical Properties of Soil at the Experimental Sites[a]

	clay (%)	silt (%)	sand (%)	OM (g kg^{-1})	CEC (cmol kg^{-1})	pH (H_2O) 1:2.5	bulk density (g cm^{-3})
trial I, 2008−2009	14.05	51.78	34.17	18.68	19.89	7.96	1.19
trial II, 2008−2009	12.34	39.89	47.77	14.25	19.59	7.77	1.04
trial III, 2009−2010	16.81	51.52	31.67	13.99	11.33	8.03	1.06

[a]Abbreviations: OM = organic matter; CEC = cation exchange capacity.

Table 2. Fumigants Used, Rates, and Application Methods Used in Trials in Mancheng, China, in 2008−2010[a]

trial	fumigant and formulation	rate (g m^{-2})	application method[b]	abbreviation of treatment
trial I, 2008−2009	Pic liquid	40	broadcast injection	Pic inj 40
	Pic gel cap	25	broadcast insertion	Pic gel cap 25
	Pic gel cap	40	broadcast insertion	Pic gel cap 40
	MB gas	50	broadcast hot gas	MB 50
	untreated control			
trial II, 2008−2009	Pic liquid	35	bed injection	Pic inj 35
	Pic liquid	50	broadcast injection	Pic inj 50
	Pic gel cap	25	bed insertion	Pic gel cap 25
	Pic gel cap	35	bed insertion	Pic gel cap 35
	MB gas	50	broadcast hot gas	MB 50
	untreated control			
trial III, 2009−2010	Pic liquid	35	broadcast injection	Pic inj 35
	Pic gel cap	35	broadcast insertion	Pic gel cap 35
	MB gas	50	broadcast hot gas	MB 50
	untreated control			

[a]Abbreviations: Pic = chloropicrin; Pic inj = chloropicrin applied by injection; Pic gel cap = chloropicrin gelatin capsule; MB = methyl bromide. [b]Broadcast fumigation: the fumigant was applied to the entire plot and strawberry beds were formed after fumigation. Bed fumigation: the fumigant was applied to preformed beds. Injection: the fumigant was injected into soil using manual injection equipment. Insertion: gelatin capsules containing the fumigant were manually inserted into the soil. Hot gas: liquid methyl bromide (from pressurized cylinders) was vaporized in a heat exchanger and then dispersed under plastic film via a plastic tube perforated with microholes.

Table 3. Dates of Fumigation and Evaluation

	date of fumigation	sampling after treatment	transplanting	weed evaluation	plant growth evaluation[a]	harvest period	root disease and plant weight evaluation
trial I, 2008−2009	Aug 16, 2008	Aug 27, 2008	Sept 7, 2008	Oct 7, 2008	Dec 30, 2008	Jan 29, 2009−March 11, 2009	March 12, 2009
trial II, 2008−2009	Aug 12, 2008	Aug 27, 2008	Sept 10, 2008	Oct 6, 2008	Jan 14, 2009	Dec 15, 2008−Feb 20, 2009	March 11, 2009
trial III, 2009−2010	Aug 1, 2009	Aug 26, 2009	Sept 5, 2009		Jan 23, 2010	Feb 4, 2010−March 21, 2010	March 25, 2010

[a]Plant growth evaluation included strawberry plant height, stem diameter, and number of fruit branches.

(15 cm depth) and inserting one gel cap per hole, without any special application tools. In the case of injection treatments, Pic liquid was injected into the soil (15 cm depth) using a manual injection machine.[16] Two different application methods of Pic gel cap and Pic liquid were evaluated in our study: bed and broadcast fumigation. In broadcast fumigation, the fumigant was applied to the entire plot and strawberry beds were formed after fumigation. In bed fumigation, the fumigant was applied to preformed beds. MB was applied by the "hot gas" method using plastic tubes perforated with microholes.[16] All treated plots were covered with polyethylene film (0.04 mm thickness). Normal cultivation techniques were used in all plots. The dates of fumigation, harvest, and sampling are provided in Table 3.

Soil Sampling and Evaluation of Fungal Populations. Following removal of the polyethylene film, three soil samples were collected from the top 20 cm of each plot. The three samples were mixed together before laboratory analysis. Populations of *F. oxysporum* and *Phytophthora* spp. were determined as indicators of the relative efficacy of each treatment in controlling soilborne fungal pathogens. *F. oxysporum* and *Phytophthora* spp. were isolated according to Komada's method[17] and Masago's method,[18] respectively.

Assessment of Weed Control. The effect of treatments on the emergence of weeds was evaluated by counting the weed species in each experimental plot. The species and number of weeds were recorded for three random 1 m × 1 m quadrates per plot. The average number of weed species for each treatment provided the weed count (plants per m^{-2}).

Evaluation of Root Disease Severity. Twenty strawberry plants were picked from each plot, and the severity of strawberry root disease was assessed separately, based on a disease severity scale of 0−4, where 0 = healthy plant and root, without disease; 1 = black brown roots comprise <25% of the entire root system; 2 = 26−50%; 3 = 51−75%; and 4 = 76−100% black brown roots. The disease scores recorded for each plot were converted into disease indices (% DI) using the formula described by McKinney[19]

$$(\%)\ \mathrm{DI} = \frac{\sum (fv)}{NX} \quad (1)$$

where f = number of plants in each class, v = class value, N = number of observed plants, and X = highest value of the evaluation scale.

Assessment of Plant Growth and Yield. Twenty strawberry plants selected from each plot were measured to determine the plant height, stem diameter, and number of fruit branches. The number of dead plants was recorded as a percentage of the total plants in each plot. At the end of the crop season, the fresh weight of individual

Table 4. Trial I: Effect of Soil Fumigation Treatment on Fungal Population and Weed Density (2008−2009)[a]

	Fusarium oxysporum		*Phytophthora* spp.		shepherd's purse *Capsella bursa-pastoris* density	
treatment (broadcast)	CFU g^{-1} soil	CE (%)	CFU g^{-1} soil	CE (%)	plants m^{-2}	CE (%)
Pic inj 40	93 b[b]	77.5	256 b	73.3	80 ab	32.5
Pic gel cap 25	109 b	73.6	309 b	67.7	25 a	79.2
Pic gel cap 40	82 b	80.1	238 b	75.2	26 a	78.3
MB 50	49 b	88.1	80 b	91.6	63 ab	47.8
untreated control	413 a		958 a		120 b	

[a]Abbreviations: Pic inj = chloropicrin applied by injection; Pic gel cap = chloropicrin gelatin capsule; MB = methyl bromide; CFU = colony-forming units; CE = control effect. [b]CFU and CE data in columns are the averages of three replications. Means within columns followed by the same letter are not different (P = 0.05) according to Duncan's multiple-range test.

Table 5. Trial I: Effect of Soil Fumigation Treatment on Strawberry Plant Growth, Mortality, Root Disease, and Yield (2008−2009)[a]

treatment (broadcast)	plant height (cm)	stem diameter (cm)	fruit branch number	mortality (%)	root disease index (%)	plant weight (g)	marketable yield (ton ha^{-1})
Pic inj 40	15.2 a[b]	1.23 a	6.44 a	0 a	87.5 ab	21.1 a	42.5 a
Pic gel cap 25	15.2 a	1.27 a	5.89 a	0 a	86.0 b	22.9 a	38.0 a
Pic gel cap 40	15.6 a	1.27 a	6.33 a	0 a	88.7 ab	21.3 a	41.7 a
MB 50	15.8 a	1.23 a	5.97 a	0 a	86.5 b	22.4 a	41.9 a
untreated control	13.6 b	1.20 a	4.75 b	0.95 b	89.7 a	18.0 b	28.9 b

[a]Abbreviations: Pic inj = chloropicrin applied by injection; Pic gel cap = chloropicrin gelatin capsule; MB = methyl bromide. [b]Numbers in columns are the average of three replications. Means within columns followed by the same letter are not different (P = 0.05) according to Duncan's multiple-range test.

Table 6. Trial II: Effect of Soil Fumigation Treatment on Fungal Population and Weed Density (2008−2009)[a]

		Fusarium oxysporum		*Phytophthora* spp.		barnyard grass *Echinochloa crusgalli* density		purslane *Portulaca oleracea* density	
treatment	application method	CFU g^{-1} soil	CE (%)	CFU g^{-1} soil	CE (%)	plants m^{-2}	CE (%)	plants m^{-2}	CE (%)
Pic inj 35	bed injection	0 b[b]	100.0	576 b	55.2	8 ab	65.2	1 bc	76.5
Pic inj 50	broadcast injection	18 b	98.8	551 b	57.2	3 bc	84.8	0 c	100.0
Pic gel cap 25	bed insertion	0 b	100.0	436 b	66.1	9 ab	60.6	3 ab	47.1
Pic gel cap 35	bed insertion	2 b	99.9	611 b	52.5	4 bc	83.3	1 b	76.5
MB 50	broadcast hot gas	0 b	100.0	280 b	78.2	1 c	97.0	0 c	100.0
untreated control		1456 a		1287 a		22 a		6 a	

[a]Abbreviations: Pic inj = chloropicrin applied by injection; Pic gel cap = chloropicrin gelatin capsule; MB = methyl bromide; CFU = colony-forming units; CE = control effect. [b]CFU and CE data in columns are the averages of three replications. Means within columns followed by the same letter are not different (P = 0.05) according to Duncan's multiple-range test.

plants in each plot was determined. Yield was recorded as the weight of marketable strawberry fruit from the marked rows in each plot at each harvest.

Statistical Analysis. The control effect (CE) on pathogens after fumigation was calculated as

$$CE = (1 - P_T/P_{CK}) \times 100 \quad (2)$$

where P_{CK} = population density of pathogens in the untreated control and P_T = population density of pathogens in the treated plot.

The CE of fumigation treatments on weeds was calculated as

$$CE = (1 - W_T/W_{CK}) \times 100 \quad (3)$$

where W_{CK} = weed density in the untreated control and W_T = weed density in the treated plot.

The resulting data on fungal populations, weed emergence, plant height, stem diameter, fruit branch number, plant mortality, disease severity, and fruit yield were subjected to variance analysis (ANOVA) for each site separately, and the means were compared by Duncan's multiple-range test. To correct for heterogeneity of variance, data on fungal populations and weed counts were transformed using square root transformation.[2] The data in percentages (mortality and root disease index) were normalized with arcsine square root transformation prior to ANOVA.[5]

RESULTS

Trial I 2008−2009. Trial I found that broadcast Pic gel cap treatments had a significant effect on soilborne fungi and weeds, as shown in Table 4. Compared to the control, all treatments substantially reduced the populations of *F. oxysporum* and *Phytophthora* spp., and there was no significant difference between the gel cap method and the injection method. The Pic gel cap treatments reduced the density of shepherd's purse weeds significantly compared to the untreated control, whereas Pic injection and MB were less effective against this weed.

The fumigation treatments also improved strawberry growth, as indicated by the data in Table 5. All treatments significantly increased plant height, plant weight, and number of fruit branches compared to the control. However, stem diameter was not significantly affected by the treatments. Dead plants occurred only in the untreated control plots. Compared to the control, all fumigant treatments significantly increased the

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Table 7. Trial II: Effect of Soil Fumigation Treatment on Strawberry Plant Growth, Mortality, Root Disease, and Yield (2008−2009)[a]

treatment	application method	plant height (cm)	stem diameter (cm)	fruit branch number	mortality (%)	root disease index (%)	plant weight (g)	marketable yield (ton ha^{-1})
Pic inj 35	bed injection	15.6 ab[b]	1.33 ab	6.70 ab	0 a	87.3 a	24.6 ab	28.7 ab
Pic inj 50	broadcast injection	16.2 a	1.33 ab	6.43 bc	0 a	82.7 a	22.8 abc	31.1 a
Pic gel cap 25	bed insertion	16.3 a	1.43 a	7.07 a	0 a	81.8 a	27.2 a	28.1 ab
Pic gel cap 35	bed insertion	16.1 a	1.40 a	6.88 ab	0 a	82.7 a	25 ab	30.2 a
MB 50	broadcast hot gas	15.6 ab	1.37 ab	6.60 abc	0 a	84.8 a	21.5 bc	31.9 a
untreated control		14.6 b	1.23 b	6.15 c	0.23 b	88.0 a	17.9 c	24.2 b

[a]Abbreviations: Pic inj = chloropicrin applied by injection; Pic gel cap = chloropicrin gelatin capsule; MB = methyl bromide. [b]Numbers in columns are the average of three replications. Means within columns followed by the same letter are not different (P = 0.05) according to Duncan's multiple-range test.

marketable fruit yield, and no significant difference was observed among the fumigant treatments.

Trial II 2008−2009. Trial II assessed the application of Pic gel caps in preformed beds. *F. oxysporum* and *Phytophthora* spp. populations were significantly reduced by all fumigant treatments compared to the control (Table 6). All fumigants had a greater impact on *F. oxysporum* than *Phytophthora*. No significant differences were observed between the gel cap and injection methods with respect to pathogen control. The data indicated that bed fumigation with Pic gel cap may provide fungal control similar to that of Pic injection (bed and broadcast). Higher rates of Pic appeared to provide greater control of barnyard grass and purslane.

The Pic gel cap treatments significantly increased plant height, stem diameter, and plant weight compared to the untreated control and often had greater influence on these parameters than other treatments (Table 7). Dead plants were found only in the control plots. There were no significant differences among the root disease indices. The yield data indicated that bed fumigation of Pic gel cap 35 and broadcast injection of Pic 50 increased fruit yield significantly compared to the untreated control.

Trial III 2009−2010. Trial III compared broadcast applications of Pic gel cap and Pic injection at the same rate (35 g m^{-2}). The different application methods had a similar impact on *F. oxysporum* and *Phytophthora* spp., reducing the populations significantly compared to the untreated control (Table 8). All fumigation treatments provided greater control of *F. oxysporum* than of *Phytophthora*.

Table 8. Trial III: Effect of Soil Fumigation Treatment on Fungal Population (2009−2010)[a]

	Fusarium oxysporum		*Phytophthora* spp.	
treatment (broadcast)	CFU g^{-1} soil	CE (%)	CFU g^{-1} soil	CE (%)
Pic inj 35	296 b[b]	87.2	2313 b	50.7
Pic gel cap 35	211 b	90.9	2673 b	43.1
MB 50	51 b	97.8	2204 b	53.1
untreated control	2313 a		4696 a	

[a]Abbreviations: Pic inj = chloropicrin applied by injection; Pic gel cap = chloropicrin gelatin capsule; MB = methyl bromide; CFU = colony-forming units; CE = control effect. [b]CFU and CE data in columns are the averages of three replications. Means within columns followed by the same letter are not different (P = 0.05) according to Duncan's multiple-range test.

In trial III the fumigant treatments increased stem diameter and influenced several other plant growth parameters. The influences of Pic gel cap and Pic injection were similar with respect to plant height, stem diameter, number of fruit branches, and plant weight. Plant mortality was significantly higher in the untreated control plots (20.5%) compared to fumigated plots (<1−6%) (Table 9). All of the fumigant treatments resulted in significantly higher yields than the untreated control. The Pic gel cap and Pic injection treatments provided statistically similar marketable yields.

DISCUSSION

This study demonstrated that Pic gel cap can reduce the populations of *F. oxysporum* and *Phytophthora* spp. in soil and is partially effective against weeds, similar to Pic injection. *F. oxysporum* seemed to be more sensitive to fumigants than *Phytophthora* spp. in these trials. Both of the Pic formulations (gel cap and liquid injection) can enhance strawberry plant growth and increase the marketable yields significantly. The results confirmed that Pic has considerable utility as a stand-alone fumigant for strawberry production.[9] Other authors have noted that Pic can also be used in combination with 1,3-dichloropropene or metham sodium to control a wide spectrum of plant pathogens and pests.[1,5]

Our results indicated that bed fumigation using Pic gel cap can control soilborne diseases to the same extent as bed and broadcast Pic injection treatments (Table 6). Duniway[9] reported that bed fumigation with Pic can also provide effective control of *Verticillium* wilt. Gilreath et al.[20] reported that bed application of a mixture of 1,3-dichloropropene and Pic (C-35) can provide soilborne pest control similar to broadcast application. Our trials found that bed fumigation using Pic gel cap improved strawberry plant growth and increased fruit yields significantly (Table 7). According to our results, the recommended application rates of Pic gel cap are 25−40 g m^{-2} for broadcast fumigation and 25−35 g m^{-2} for bed fumigation. Bed fumigation generally resulted in the application of less fumigant per hectare than broadcast fumigation because of reduced areas needed for treatment.

Pic is a severe lachrymator and requires personal protective equipment when injected in liquid form, due to its pungent odor. This is a limitation to its adoption in China because most farmers apply fumigants themselves without essential application tools and personal protection equipment. Pic gel cap is a new formulation that has little offensive smell due to the encapsulation of the Pic liquid in a gelatin shell; it reduces the

Table 9. Trial III: Effect of Soil Fumigation Treatment on Strawberry Plant Growth, Mortality, Root Disease, and Yield (2009−2010)[a]

treatment (broadcast)	plant height (cm)	stem diameter (cm)	fruit branch number	mortality (%)	root disease index (%)	plant weight (g)	marketable yield (ton ha^{-1})
Pic inj 35	11.7 a[b]	1.39 a	7.85 a	5.95 b	71.7 ab	21.9 ab	33.4 a
Pic gel cap 35	12.3 a	1.38 a	7.58 ab	2.38 bc	65.4 b	22.5 ab	37.6 a
MB 50	14.2 a	1.44 a	8.63 a	0.48 c	64.2 b	25.4 a	33.5 a
untreated control	11.7 a	1.31 b	6.53 b	20.5 a	76.7 a	16.1 b	25.4 b

[a]Abbreviations: Pic inj = chloropicrin applied by injection; Pic gel cap = chloropicrin gelatin capsule; MB = methyl bromide. [b]Numbers in columns are the average of three replications. Means within columns followed by the same letter are not different ($P = 0.05$) according to Duncan's multiple-range test.

risk of exposure to workers and bystanders during fumigation. In addition, Pic gel cap formulations are easy for farmers to handle and can be applied without special training or protective equipment. Moreover, the use of Pic gel cap could also reduce Pic emissions to the atmosphere.[15]

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Author Contributions

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Notes

The authors declare no competing financial interest.

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