Control of Soilborne Pathogens of *Zingiber officinale* by Methyl Iodide and Chloropicrin in China

Yuan Li, Lida Chi, Liangang Mao, Dongdong Yan, Zhuanfang Wu, Taotao Ma, Meixia Guo, Qiuxia Wang, Canbin Ouyang, and Aocheng Cao, Department of Pesticides, Key Laboratory of Pesticide Chemistry and Application, State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing, 100193

Abstract

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Development of effective alternative soil fumigants is essential to the phasing out of methyl bromide (MeBr) while keeping major soilborne pathogens under control. Here, we report on the laboratory studies and field trials evaluating methyl iodide (MeI) and chloropicrin (Pic) for control of major soilborne ginger (*Zingiber officinale*) pathogens *Ralstonia solanacearum*, *Pythium* spp., *Fusarium oxysporum*, and *Meloidogyne incognita* in Shandong province of China. Laboratory studies indicated that MeI at 24 mg/kg of soil was most effective, reducing four pathogens by >90%. Treatments with MeI+Pic at 12 mg/kg (1:3 and 1:5) also reduced these pathogens by >82%. In the field trials, MeI

at 30 or 40 g/m² and MeI+Pic (1:3) at 40 g/m² yielded excellent long-term control of all target pathogens. These treatments allowed ginger plants to maintain vigorous growth and produce a greater number of tillers (>12 per plant), and increased ginger yields by >80% compared with the nontreated controls. MeI at a reduced rate of 20 g/m² or Pic at 40 g/m² provided levels of disease control similar to MeBr. These studies demonstrated that injection treatments with MeI at 30 and 40 g/m², and MeI+Pic (1:3) at 40 g/m², followed by covering with virtually impermeable film, are effective alternatives of soil fumigation for control of the major ginger pathogens in Shandong.

Ginger (*Zingiber officinale* Roscoe) is one of the major commercial crops in Shandong Province, China, where there is a large ginger producer with a long planting history. Owing to successive plantings over many years, soilborne diseases of ginger have become increasingly serious, leading to severe losses in ginger yield and quality. The main soilborne pathogens are *Ralstonia solanacearum*, *Pythium* spp., *Fusarium oxysporum*, and *Meloidogyne incognita* (19). These diseases are difficult to control. Local farmers often use methyl bromide (MeBr) to disinfest soil prior to planting. Therefore, Shandong is a large consumer of MeBr in China. However, MeBr is due to be phased out in developing countries by 1 January 2015 (3). The planned withdrawal of MeBr from use as an agricultural fumigant has prompted a great deal of research to develop economically viable alternatives (6,22,28,29,35).

Among the soil fumigants presented by The Methyl Bromide Technique Option Committee (32) is methyl iodide (MeI). MeI (CH₃I) is liquid at room temperature, with a boiling point of 43°C; it may persist in the atmosphere for 2 to 8 days but degrades by photolysis before reaching the ozone layer. Thus, it is relatively safe for the ozone layer, with an ozone-depleting potential of 0.016/m³, which is 1/40th that of MeBr (10,13,14,32). In recent years, many scholars have investigated the use of MeI as a new fumigant for controlling soilborne diseases and insects, as well as its movement in soil (21). Their results indicated that MeI is quite effective for controlling nematodes and *Fusarium* spp. under laboratory conditions. Some studies have evaluated the application of MeI to strawberry plants (7) and peach trees (9) for control of insect-transmitted diseases. MeI also was used to control *M. incognita* in soils (2,15). Ashworth et al. (1) reported that MeI has been increasingly used as an alternative to MeBr. However, there was no information on MeI for control of soilborne diseases of ginger crops.

The objective of the present study was to determine the efficacy of MeI alone and in combination with chloropicrin (Pic) as a soil fumigant for control of soilborne pathogens of ginger under laboratory and field conditions.

Materials and Methods

Two sets of field experiments were conducted during 2010 in two commercial fields in Anqiu, Shandong Province, China. Anqiu has been an important ginger production area in China for more than 15 years but faces problems caused by heavy infestations by root-knot nematodes, soilborne fungi, and bacteria. The fields of study had been severely affected by soilborne diseases, and the yield losses had been particularly severe. The field experiments were accompanied by laboratory studies on the activity of fumigants against soilborne pathogens in ginger-cultivation soils.

Effects of soil fumigation with MeI or Pic in the laboratory assays. Two fumigants—MeI (Beijing Chemical Regents Co., Ltd.) and Pic (Dalian Lveng Chemical Co., Ltd.)—were used in this laboratory study. They were evaluated singly and in combination. Treatments included MeI alone at rates of 2, 3, 6, 12, and 24 mg/kg of soil; Pic alone at rates of 9 and 10 mg/kg of soil; MeI+Pic at ratios of 2 + 10 (1:5) and 3 + 9 (1:3) mg/kg of soil; plus a nontreated control. Each treatment had three replicates.

Soil samples were collected from the top 20 cm of a ginger field heavily infested by nematodes and soilborne pathogens in Anqiu. The soil was composed of 59.84% sand, 37.43% silt, and 2.73% clay, with an organic matter content of 12.21 mg/kg and pH at 5.42. The soil was sieved through a 2-mm mesh and then mixed well. The soil moisture was 12.22% (wt/wt). Particle size analyses were performed using the pipette method (30). Organic carbon contents were determined by wet oxidation, using the method described by Nelson et al. (26). Soil was mixed with H₂O at a ratio of 1:2.5 before the pH reading was taken. Soil moisture contents were measured by drying to constant mass in an oven at 105 ± 5°C for 5 min.

Approximately 600 g of soil was placed into each of 30 1.5-liter desiccators. After the soils were mixed well in each desiccator, a fumigant was injected into the soil with a pipette and the desiccators were immediately closed with covers. The desiccators were placed in incubators at 25°C for 5 days. The desiccators were then opened for a day to release the residual fumigants.

Corresponding author: A. Cao, E-mail: caoac@vip.sina.com

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At the termination of assays, four major soilborne pathogens (*R. solanacearum*, *F. oxysporum*, *Pythium* spp., and *M. incognita*) were isolated from the soil and quantified by counting colonies or numbers of individuals, and the fumigation efficacy of MeI and MeI+Pic was subsequently calculated. *R. solanacearum* was isolated from the soil and quantified using triphenyl tetrazolium chloride culture medium (17). *F. oxysporum* was isolated and quantified using the method of Komada (18), and *Pythium* spp. were isolated according to Martin's method (23). Root-knot nematodes *M. incognita* were extracted and quantified by centrifugation-based methods, as described by Liu (20). The colonies of *R. solanacearum*, *F. oxysporum*, and *Pythium* spp. were counted directly from the agar plates. The dead and live nematodes were counted under a dissecting microscope. A nematode that was not moving was touched lightly with a dissecting needle; if it did not move at all, it was counted as dead; otherwise, counted as living.

Field efficacy trials of MeI and Pic. Two trials (I and II) were carried out in 2010 in Xirulin Village, Linghe Town, Anqiu City, Shandong Province. The trial I site was 2 km away from the trial II site. The trials were located at latitude 36.25', longitude 119.12', which has a temperate climate with a yearly average temperature of 12.2°C, total precipitation of 646.3 mm, and illumination of 2,502.1 h. The soil texture of the experimental fields was sandy loam with an organic content of 12.21 mg/kg, pH 5.42, unit weight of 1.50 g/cm³, and water content of 12.22%. Ginger had been cultivated in both fields for more than 15 years, and both were heavily infested with soilborne pathogens.

MeI and MeI+Pic were used in both trials. MeBr (Israel Dead Sea Bromine Products Co., Ltd.) was used as a control fumigant. To determine the best dosage of MeI for application, MeI was assessed singly at three rates of 20, 30, and 40 g/m², and in combination with Pic at two ratios of 1:5 and 1:3, both mixtures applied at 40 g/m². MeBr at 40 g/m² and Pic at 40 g/m² were used as controls (Table 1). Each treatment had three replicates. The same treatments were evaluated in both trials. Each treatment plot area in both experimental fields was 28 m². All

treatments were covered with virtually impermeable film (VIF) mulch (Bromostop, Bruno Rimini, Ltd.). MeI, MeI+Pic, and Pic treatments were applied by dynamic injection using a small drug delivery machine (Model DJR-202, Dalian Jinmei Soil Disinfection Equipment Development Co., Ltd.). MeBr was applied by the hot gas method (7). After application of the fumigants, the treated regions were immediately covered with VIF. Nontreated control microplots were cultivated, tamped, and sealed with VIF but received no fumigant. Fumigation treatments started on 17 March 2010, and lasted for 2 weeks.

After 2 weeks of fumigation, the tarp was removed for 1 day and soil samples from the two trials were collected and analyzed in the laboratory. Soil samples were collected from the 0- to 20-cm soil layers in each treated and nontreated plot. During the ginger harvest period, soil samples were collected and analyzed once again. Soilborne bacteria, fungi, and nematodes in the sampled soils were determined separately using the same methods described above. *R. solanacearum*, *F. oxysporum*, and *Pythium* spp. were isolated from the soil and quantified using the methods described by Kelman (17), Komada (18), and Martin (24), respectively. Root-knot nematodes (*M. incognita*) were extracted by centrifugation, based on the methods described by Liu (20).

The ginger transplant time was 20 April 2010. During the ginger growth period, the plots treated by soil fumigation were carefully cultivated to avoid reinfestation from the nontreated control plots. Twenty plants were selected from each plot and the height and tiller number were measured 121 days after planting. During the harvest period, the ginger yield was determined 181 days after planting to evaluate the fumigation effect.

Statistical analyses. The efficacy of treatment with a combination of MeI+Pic was calculated according to the following equation, based on the method described by Colby (4): $E - E_0 = E - X_1X_2/100$, where E is the actual measured efficacy of the combination, E_0 is the expected efficacy of the combination, X_1 is the actual measured efficacy of the first fumigant, and X_2 is the actual measured efficacy of the second fumigant. If $E > E_0$, the actions of the combined agents were synergistic; if $E < E_0$, the actions of the two agents were antagonistic.

Data were analyzed by analysis of variance using SAS (v. 8.0 for Windows). Differences among means were determined by Fisher's least significant difference test at $P = 0.05$ (5,31).

Results

Effects of soil fumigation with MeI and Pic in the laboratory assays. The effect of MeI on *R. solanacearum*, *Pythium* spp., *M. incognita*, and *F. oxysporum* is shown in Table 2. MeI at 2 mg/kg of soil had low efficacy on four pathogens (35.06 to 59.38%). At 3 or 6 mg/kg of soil, MeI demonstrated improved efficacy, with >82% against *R. solanacearum* and *M. incognita* and 57.65 to 72.5% against *F. oxysporum* and *Pythium* spp. MeI at a concentra-

Table 1. Soil fumigation treatments in field trials

Code	Treatment ^z	Rate (g/m ²)	Application method
1	MeI	20	Injection
2	MeI	30	Injection
3	MeI	40	Injection
4	MeI+Pic(1:3)	40	Injection
5	MeI+Pic(1:5)	40	Injection
6	Pic	40	Injection
7	MeBr	40	Hot gas distribution
8	Control	...	...

^z MeI = methyl iodide, Pic = chloropicrin, and MeBr = methyl bromide.

Table 2. Synergistic control activity of methyl iodide and chloropicrin on four soilborne pathogens of ginger crops^x

Treatment ^y	Rate ^z	Pathogen control (%)															
		<i>Ralstonia solanacearum</i>				<i>Fusarium oxysporum</i>				<i>Pythium</i> spp.				<i>Meloidogyne incognita</i>			
		<i>E</i>	<i>E</i> ₀	<i>E</i> - <i>E</i> ₀	CE	<i>E</i>	<i>E</i> ₀	<i>E</i> - <i>E</i> ₀	CE	<i>E</i>	<i>E</i> ₀	<i>E</i> - <i>E</i> ₀	CE	<i>E</i>	<i>E</i> ₀	<i>E</i> - <i>E</i> ₀	CE
MeI	2	59.38	...	...	...	52.5	...	...	...	35.06	...	...	...	56.4	...	...	...
MeI	3	86.65	...	...	...	71.2	...	...	...	57.65	...	...	...	82	...	...	...
MeI	6	90.83	...	...	...	72.5	...	...	...	67.01	...	...	...	86.1	...	...	...
MeI	12	92.24	...	...	...	90.6	...	...	...	89.3	...	...	...	90.9	...	...	...
MeI	24	97.3	...	...	...	92.3	...	...	...	90.35	...	...	...	92.8	...	...	...
MeI+Pic (1:3)	3 + 9	93.44	69.42	24.02	+	92.8	54.2	38.6	+	89.34	37.65	51.69	+	82.7	37.9	44.73	+
MeI+Pic (1:5)	2 + 10	91.85	50.28	41.57	+	91.3	41.2	50.1	+	86.77	24	62.78	+	88.7	53.5	35.21	+
Pic	9	80.11	...	...	...	76	...	...	...	65.31	...	...	...	65.2	...	...	...
Pic	10	84.67	...	...	...	78.5	...	...	...	68.44	...	...	...	67.3	...	...	...
Control	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...

^x E is the actual measured efficacy of the combination, E_0 was the expected efficacy of the combination, and CE = combined efficacy. If $E > E_0$, the actions of the agents were synergistic; if $E < E_0$, the actions of the agents were antagonistic; + means synergistic.

^y MeI = methyl iodide and Pic = chloropicrin.

^z Rate in milligrams per kilogram of soil.

tion of 12 or 24 mg/kg of soil showed the best efficacy against all four pathogens (>89.3%). Treatment with MeI+Pic (1:3) at 12 mg/kg showed a high control efficacy over all the pathogens tested (>88%), while that of MeI+Pic (1:5) at 12 mg/kg was 82.7%. Treatments with MeI+Pic (both 1:5 and 1:3) were equally as effective as treatments with MeI alone at 12 or 24 mg/kg of soil. MeI+Pic combination treatments were more efficacious than either fumigant used alone, indicating a strong synergistic activity. Treatments with Pic at 9 or 10 mg/kg of soil showed a control effect similar to that of MeI alone at 3 mg/kg of soil, having an efficacy of >65% against the pathogens. There was no significant difference between the effects of the two Pic concentrations. All treatments showed a better control of *R. solanacearum* than on the other three pathogens.

Field efficacy trials of MeI and Pic. Significant control of all four pathogens was observed in all soil fumigation plots immediately after treatments in both field trials, with MeBr as the best performer (Fig. 1). The results obtained in the two field trials were similar. In trials I and II, all fumigation treatments except MeI at

20 g/m² provided great control of *M. incognita* and *R. solanacearum*. Plots treated with MeI at 30 to 40 g/m² and MeI+Pic (1:3) at 40 g/m² had significantly lower densities of *R. solanacearum* (<280 CFU per 10 g of soil) and *M. incognita* (<50 nematodes per 10 g of soil) compared with the nontreated controls (>1,700 CFU per 10 g of soil on *R. solanacearum* and >300 nematodes per 10 g of soil on *M. incognita*). MeI+Pic (1:5) and Pic at 40 g/m² provided a similar level of control.

Likewise, all fumigation treatments reduced the population densities of *Pythium* spp. and *F. oxysporum*. MeI at 30 and 40 g/m² and MeI+Pic (1:3) at 40 g/m² reduced the population densities of *Pythium* spp. and *F. oxysporum* to <500 CFU per 10 g of soil while the nontreated controls had >1,200 CFU per 10 g of soil. MeI at 20 g/m², MeI+Pic (1:5), and Pic at 40 g/m² provided similar levels of control for *Pythium* spp. and *F. oxysporum* but less control than MeBr.

This pathogen suppression continued throughout the growing season in both trials, as indicated by soil assays performed at ginger harvest time (Table 3). All treatments reduced *R. solanacearum*

Fig. 1. Recovery of four soilborne pathogens from soil samples collected in the field trials immediately after fumigation treatments. MeI = methyl iodide, Pic = chloropicrin, MeBr = methyl bromide, and CK = nontreated control.

Table 3. Effects of soil fumigation on four soilborne pathogens in soil at the ginger harvest^y

Treatments ^z	<i>Ralstonia solanacearum</i>		<i>Pythium</i> spp.		<i>Fusarium oxysporum</i>		<i>Meloidogyne incognita</i>	
	CFU/g soil	CE (%)	CFU/g soil	CE (%)	CFU/g soil	CE (%)	N	CE (%)
Trial I								
MeI 20 g/m ²	490 b	76.13	527 b	60.47	544 b	61.47	81 b	63.68
MeI 30 g/m ²	302 b	85.29	299 bc	77.57	267 cd	81.82	23 c	89.69
MeI 40 g/m ²	286 b	86.07	255 c	80.87	112 d	93	20 c	91.03
MeI+Pic (1:3) 40 g/m ²	198 b	90.36	198 c	85.15	334 c	77.15	40 bc	82.06
MeI+Pic (1:5) 40 g/m ²	247 b	87.97	322 bc	75.84	422 c	71.24	67 bc	70.92
Pic 40 g/m ²	279 b	86.41	525 b	60.47	553 b	61.02	94 b	57.85
MeBr 40 g/m ²	126 b	93.86	202 c	84.85	153 d	89.65	21 c	90.58
Control	2,053 a	...	1,333 a	...	1,478 a	...	223 a	...
Trial II								
MeI 20 g/m ²	531 b	76.75	401 b	60.09	893 b	62.78	69 bc	71.72
MeI 30 g/m ²	279 b	87.78	301 b	70.01	534 b	77.75	48 c	80.33
MeI 40 g/m ²	106 b	95.36	250 b	75.10	582 b	75.75	21 c	91.39
MeI+Pic (1:3) 40 g/m ²	148 b	93.52	254 b	74.70	472 b	80.33	34 c	86.07
MeI+Pic (1:5) 40 g/m ²	104 b	95.45	320 b	68.13	526 b	78.08	91 b	62.70
Pic 40 g/m ²	229 b	89.97	361 b	64.04	615 b	74.38	102 b	58.20
MeBr 40 g/m ²	99 b	95.67	251 b	75.00	471 b	80.38	19 c	92.21
Control	2,284 a	...	1,004 a	...	2,400 a	...	244 a	...

^y In each column, data were the means of three replicates. Means followed by the same letter were not different according to the least significant difference test at *P* = 0.05.

^z MeI = methyl iodide, Pic = chloropicrin, and MeBr = methyl bromide.

by >75%. Similarly, MeI at 30 and 40 g/m² and MeI+Pic (1:3) at 40 g/m² reduced *F. oxysporum* and *Pythium* spp. by >70%, which was similar to results with MeBr. These treatments also reduced nematodes by >80%, which was slightly less than that achieved with MeBr.

Greater number of tillers, taller plants, and higher ginger yield were observed in all fumigant-treated plots than the nontreated control plots (Tables 4 and 5). Plots treated by MeBr produced ginger at 99,346.4 kg/ha in trial I and 98,947.2 kg/ha in trial II, a 90% yield increase compared with the controls. The plots treated with MeI at 30 and 40 g/m² and MeI+Pic (1:3) at 40 g/m² had a greater number of tillers, approximately 12 per plant, and resulted in >80% of ginger yield compared with those treated with MeI or Pic at other rates. Plots treated with MeI at 20 g/m² produced about 10 tillers per plant and ginger at 80,000 kg/ha, which was still significantly higher than in the nontreated controls.

Discussion

This study demonstrated that MeI alone and in combination with Pic can effectively control four major soilborne ginger pathogens: *R. solanacearum*, *Pythium* spp., *F. oxysporum*, and *M. incognita*.

In laboratory studies, MeI provided better control of *R. solanacearum* and *M. incognita* while Pic showed better control of *R. solanacearum* and *F. oxysporum*. Treatments with MeI at different concentrations all showed better control of *R. solanacearum* and *M. incognita* than of *F. oxysporum* and *Pythium* spp., with their effectiveness depending on the dose used. Our results with the four pathogens and data in other reports (16,33) show that the combination of MeI and Pic has markedly high control efficacy on soilborne pathogens owing to its broader spectrum of activity and synergism between the two compounds. Pic-only treatments showed better efficacy in the control of *R. solanacearum* and *F. oxysporum* than of *Pythium* spp. and *M. incognita*, probably because Pic is primarily used as a fungicide and is expected to have a greater effect in the control of fungi than of nematodes (8,12,16,25).

Field trials along with the lab studies provided effective alternatives to MeBr for control of the major soilborne ginger pathogens in Shandong province of China. MeI at 30 and 40 g/m² and MeI+Pic (1:3) at 40 g/m² provided levels of long-term control similar to MeBr for soilborne bacteria, fungi, and nematodes. As a

result, plants of these treatments maintained vigorous growth, had a greater number of tillers, and increased yield by >80% compared with the nontreated control. These results are in agreement with those from previous studies that used MeI+Pic (50:50 mixtures) to control the soilborne pathogen *Verticillium dahlia* on strawberry (16). MeI at 30 to 40 g/m² and MeI+Pic (1:3) at 40 g/m² were appropriate dosages for the economic and efficient fumigation of ginger fields. In both field trials, the effectiveness of fumigation was good in the 0- to 20-cm soil layers, probably owing to high penetration by MeI and MeBr (7,21,32). In addition, the odor and eye irritation caused by Pic at very low levels is an effective alert for the presence of this compound and has been used previously for this purpose along with MeBr (7,16,24). Considering the high cost of MeI and the relatively low cost of Pic, it is also good to use a mixture of MeI and Pic to reduce chemical costs while maintaining high efficacy.

Fumigant injection is a viable method of soil treatment. Duniway et al. (7) reported that MeI is generally applied by conventional shank methods and drip systems (7,27). However, we used the injection method for MeI and MeI+Pic and obtained good field efficacy. In addition, this mechanical injection was simple to implement, saving time, human resources, and cost, and is more suitable for large-scale ginger field fumigation. The volatile and toxic nature of MeI has drawn wide attention because of concerns over potential atmospheric emissions following field fumigation treatment (34). Ashworth et al. (1) reported that surface tarping with VIF would be highly effective as an emission-reduction strategy and would also result in maintenance of high soil gas concentrations (which is important for pest control). In our study, surface tarping with VIF was effective for the control of soilborne pathogens and produced high ginger yields.

In conclusion, this study demonstrated that MeI at 30 to 40 g/m² and MeI+Pic (1:3) at 40 g/m², applied by injection treatment and covered with VIF tarps, are effective alternatives to MeBr in soil fumigation for ginger production in Shandong province of China.

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Table 4. Effect of soil fumigation on ginger plant height, tiller number, and yield in trial I^y

Treatment ^z	Plant height (cm)	Tillers/plant	Yield (kg/ha)	Yield increase (%)
MeI 20 g/m ²	70.3 b	10.1 b	86,853.6 ab	66.55
MeI 30 g/m ²	74.7 ab	12.8 ab	94,137.5 a	80.52
MeI 40 g/m ²	79.9 a	15.4 a	94,428.6 a	81.08
MeI+Pic (1:3) 40 g/m ²	82.7 a	15.1 a	95,542.9 a	83.21
MeI+Pic (1:5) 40 g/m ²	78.3 a	10.3 b	80,407.1 b	54.19
Pic 40 g/m ²	72.4 ab	10.7 b	81,365.1 b	56.03
MeBr 40 g/m ²	80.2 a	14.9 a	99,346.4 a	90.51
Control	58.3 c	7.3 c	52,148.2 c	...

^y In each column, data were the means of three replicates. Means followed by the same letter were not different according to the least significant difference test at *P* = 0.05.

^z MeI = methyl iodide, Pic = chloropicrin, and MeBr = methyl bromide.

Table 5. Effect of soil fumigation on ginger plant height, tiller number, and yield in trial II^y

Treatment ^z	Plant height (cm)	Tillers/plant	Yield (kg/ha)	Yield increase (%)
MeI 20 g/m ²	71.2 b	10.5 ab	87,029.7 ab	69.95
MeI 30 g/m ²	71.9 b	11.8 ab	94,418.6 a	84.38
MeI 40 g/m ²	79.7 a	12.3 ab	95,314.5 a	86.13
MeI+Pic (1:3) 40 g/m ²	80.4 a	14.5 a	96,718.3 a	88.87
MeI+Pic (1:5) 40 g/m ²	78.0 a	14.0 a	82,115.8 b	60.36
Pic 40 g/m ²	70.3 b	10.6 ab	82,505.4 b	61.12
MeBr 40 g/m ²	79.6 a	13.9 a	98,947.2 a	93.23
Control	61.2 c	7.1 b	51,207.6 c	...

^y In each column, data were the means of three replicates. Means followed by the same letter were not different according to the least significant difference test at *P* = 0.05.

^z MeI = methyl iodide, Pic = chloropicrin, and MeBr = methyl bromide.

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