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Biological Control 36 (2006) 216–223
Biological Control
www.elsevier.com/locate/ybcon

Evaluation of biocontrol efficiency of different *Bacillus* preparations and field application methods against Phytophthora blight of bell pepper

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Received 24 May 2005; accepted 31 October 2005
Available online 7 December 2005

Abstract

Biocontrol efficiency of various *Bacillus* preparations (BB11 and FH17 strains, and a mixture of both strains (BF) at a 1:1 ratio by concentration) and different application methods against Phytophthora blight of bell pepper were studied. The application methods included (A) mixing (mixing BF with rapeseed residue and then immediately applying in the field), (B) composting (mixing BF with rapeseed residue and made compost before application), (C) spraying (spraying diluted BF into field or rhizosphere of plants), and (D) watering (watering diluted BF into field or rhizosphere of plants). In greenhouse experiments, the addition of BF increased biocontrol efficiency (60.3%), and yield increase (200%) was better than with BB11 (55.8 and 80.6%, respectively) or FH17 (37.1 and 50.0%, respectively). In field trials at Huai'an in 2001, the best dosages of BF mixture (10^{10} cfu/ml) with the four above-mentioned application methods were 15, 7.5, 15, and 22.5 L/ha, respectively. When preparations were applied at the best dosage in the same field, the BF mixture provided superior biocontrol efficiency and greater yield increase with treatment B than those with treatment A or C. Combining the field trial results from 2002 to 2003 at Huai'an and Wu'han, the total average control efficiencies and yield increases for treatments A, B, and C reached 81.0, 88.0, and 79.1% and 33.1, 44.3, and 29.1%, respectively, with their best dosages. However, method B, composting, provided better disease control and greater yield increases than all other methods, and did so at a lower application rate.

Keywords: *Bacillus*; *Phytophthora capsici*; Biocontrol; Application method

1. Introduction

Phytophthora blight of bell pepper, caused by *Phytophthora capsici* Leonian, is a worldwide disease of economic significance (Babadoos, 2000; Erwin and Ribeiro, 1996; Hwang and Kim, 1995; Lee et al., 2001; Leonian, 1922; Ristaino and Johnston, 1999; Tamietli and Valentino, 2001). This soil-borne disease is very difficult to control, partly due to the pathogen's ability to survive for several years as oospores in soil or as mycelium in plant residues (Lamour and Hausbeck, 2001). These are the primary forms of inocula in soil, and levels of this inoculum can increase each year with occurrence of the disease. The disease cycle is very short, only 3–5 days from initial infection to sporulation (Zheng, 1997), and the pathogen disperses rapidly by splashing rain as well as flowing irrigation and surface water, especially under the condition of wet soils above 18 °C and prolonged wet periods with air temperatures in the 24–29 °C range (Zitter, 1989). Once disease breaks out, it causes massive losses despite spraying with fungicides. Furthermore, pathogens become resistant to fungicides if applied continually (Hausbeck et al., 2001). Therefore, the best tactic is to reduce initial inoculum by soil treatment prior to transplanting and

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doi:10.1016/j.biocontrol.2005.10.012

prevent disease outbreak. Chemicals used to treat soil, such as methyl bromide, are normally toxic. In addition to other potential health, safety, and environmental hazards, methyl bromide is classified as an ozone-depleting compound and is scheduled for removal from world markets by 2015 (Larkin and Fravel, 1997). Owing to advantages in environmental and health safety, many biocontrol agents or their metabolites suppressive to *Phytophthora* have been studied [e.g., *Trichoderma harzianum* Rifai, *Bacillus subtilis* (Ehrenberg) Cohn, *Gliocladium virens* Miller, Giddens, and Foster, *Streptomyces griseoviridis* Anderson, and phenazine-1-carboxylic acid (Anandaraj and Sarma, 1994; Lee et al., 2003; Namec et al., 1996)]. Current biological agents on the market, such as *T. harzianum* and *T. viride* Pers.:Fr., Polyversum (produced by Biopreparaty, Czech Republic; the active ingredient of biopreparation Polyversum is *Pythium oligandrum* Drechs. which colonizes the rhizosphere of treated plants and functions through mycoparasitism and competition) or Mycostop MIX (produced by Verdera Oy; Finland and its active ingredients are dried spores and mycelium of *S. griseoviridis* Anderson Strain K61), are usually incorporated into the soil, sprayed, or injected, according to the manufacturers' instructions. Their suppressive mechanisms include inducing plant systemic resistance (Khan et al., 2004; Yan et al., 2002) and antagonism (Qiu et al., 2004), among others. The suppressive effect of many biocontrol agents has been studied, including their repetitive effect (Steddom et al., 2002), but results normally involved a particular application method, while few studies examined the biocontrol effects of one agent under different application methods.

Several *Pseudomonas* and *Bacillus* spp. have been screened as potential biocontrol agents against *Ralstonia solanacearum* Smith, by inhibition zone and root-colonizing capacity in pepper (Guo et al., 1996, 2001). A total of 45 isolates showed antagonistic effects on *R. solanacearum.* Among them, six isolates, including BB11 and FH17 from rhizospheres of ginger and pepper, respectively, showed comparatively better colonization and biocontrol effects against bacterial wilt of pepper in field trials at Huai'an (Guo et al., 2001). BB11 and FH17 were identified as *Bacillus* spp. and later found to be also antagonistic to *P. capsici* through the inhibition-zone method, and there was no inhibition effect between BB11 and FH17. Therefore, BB11 and FH17 were mixed, at a ratio of 1:1 by concentration (BF), and biocontrol efficiency evaluated for different application methods against Phytophthora blight of bell pepper.

2. Materials and methods

2.1. Preparation of BF mixture, pathogen inoculum, and plants

At Huai'an, bacterial strains BB11 and FH17 were isolated from rhizosphere of ginger (*Zingiber officinale* Rose) and bell pepper (*Capsicum annuum* L. var. grossum Seudt.), respectively, and both were used to control bacterial wilt caused by *R. solanacearum* (Guo et al., 2003, 2004). They were cultured separately in potato sucrose broth (potato 200 g, sucrose 20 g, water 1000 ml, pH 8.0) in a 10-L fermenter and then mixed at a ratio of 1:1 (v/v, 1×10^{10} cfu/ml), as the BF mixture preparation. The bacterial concentration of the BF mixture was adjusted to 1×10^{10} cfu/ml. Inocula were prepared from a single, strongly pathogenic strain BP05 of *P. capsici*, which was isolated from infected bell pepper in Huan'an in 1999. BP05 was grown on V8 juice agar in the dark for 2 weeks at 25 °C and maintained at 4 °C for 2 h to stimulate zoospore release (Sujikowski et al., 1999). Zoospores were harvested, counted using a hemocytometer, and the concentration adjusted to 5.0×10^4 zoospores/ml. The bell pepper used in the experiments was Bian'jiao No.1, produced by the Tian'jin Vegetable Institution.

2.2. Greenhouse experiment

Eighteen-day-old bell pepper seedlings were soaked in a 10^{10} cfu/ml suspension of either the BF mixture or individual *Bacillus* strains for 30 min after being carefully pulled out of the seedling tray with soil around the roots. They were then planted into bigger plastic pots (22 cm diameter) containing sterile organic soil or soaked in sterile water as control. Each group had 24 plants and each plant was transplanted into one pot. All plants were placed in a darkened chamber, where moisture was greater than 90% RH and room temperature was 28 °C, for 2 h before inoculation in the early evening. Both sides of the foliage were then sprayed with about 3 ml of zoospore suspension per plant using a household compression sprayer (SX-573; Shi-Xia Sprayer, Zhe-Jiang Province, China). Plants were kept in the growth chamber overnight and then transferred to the greenhouse. The disease incidence was recorded 60 days after inoculation.

2.3. Evaluation of field application methods

In field trials, four application methods of the biocontrol preparation were studied: (A) mixing, (B) composting, (C) spraying, and (D) watering. Due to space limitations, each application method was conducted in a different greenhouse at Huai'an in the autumn of 2001. All treatments had four replicates and each plot was 20 m^2 with 120 plants. Plots were arranged randomly and separated by three blank ridges.

The mixing treatment consisted of spraying BF mixture (3, 7.5, 15, or 22.5 L/ha, 10^{10} cfu/ml), diluted with water (4000 L/ha), onto rapeseed residue (2250 kg/ha) and mixed evenly. The residue was scattered on the plots and each plot ploughed separately. The field was then covered with a plastic film and the plants were transplanted after 7 days. The original suspension volumes used in this experiment were: mixing 1, 3; mixing 2, 7.5; mixing 3, 15; and mixing 4, 22.5 L/ha. The temperature under the membrane was 15–35 °C.

The composting treatment consisted of mixing BF mixture (3, 7.5, 15, or 22.5 L/ha, 10^{10} cfu/ml), diluted with water (4000 L/ha), with rapeseed residue (2250 kg/ha), and mixed evenly. The residue was piled up, covered with black plastic film to improve temperature in favor of the BF mixture and agitated twice daily to avoid extremely high temperatures during the 3-day natural fermentation process. The composts' humidity was kept between 70 and 90% and temperature between 25 and 50 °C. The fermented compost was scattered on the plots and each plot ploughed separately. The field was then covered with a plastic film and the plants were transplanted after 7 days. The original suspension volumes used in this experiment were: composting 1, 3; composting 2, 7.5; composting 3, 15; and composting 4, 22.5 L/ha. The temperature under the membrane was 15–35 °C.

The spraying treatment consisted of spraying BF mixture (3, 7.5, 15, or 22.5 L/ha, 10^{10} cfu/ml), diluted with water (4000 L/ha), onto four plots where rapeseed residue (2250 kg/ha) had been evenly scattered and ploughed into the soil. The field was then covered with a plastic film and the plants were transplanted after 7 days. The original suspension volumes used in this experiment were: spraying 1, 3; spraying 2, 7.5; spraying 3, 15, and spraying 4, 22.5 L/ha. The temperature under the membrane was 15–35 °C.

The watering treatment consisted of water BF mixture (3, 7.5, 15, or 22.5 L/ha, 10^{10} cfu/ml), diluted with water (4000 L/ha), onto the pepper rhizosphere immediately after transplanting. The original suspension volumes used in this experiment were: watering 1, 3; watering 2, 7.5; watering 3, 15; and watering 4, 22.5 L/ha. BF mixture was hand watered and not flooded with a hose. Since this watering method is very laborious and unsuitable for general use, it was discontinued in the field experiments at Huai'an and Wu'han in 2002 and 2003. The soil had already been treated with rapeseed residue (2250 kg/ha) before transplanting.

There were two controls: (1) ferment control, 135 kg/ha ferment (a commercially available product in China, manufactured by Da'hua Compost, Jiang'su Province) was mixed with 2250 kg rapeseed residue and then treated in the same way as composting treatment; (2) water control, no treatment. The plots were simply watered; the same amount of rapeseed residue was used before transplanting.

The biocontrol efficiencies were calculated by disease index, and fruit production was determined by adding up three harvests each plot, with fruits of all sizes picked and counted. Mixing, composting, spraying, and watering treatments were done in four large separate plastic houses at the same location. Each house had one water control treatment. Each test consisted of four rate treatments and a water control. Each treatment was replicated four times. Each experiment had a completely randomized design. In the composting treatment, a water and a ferment control were included to compare the effect of BF mixture with a commercially available biocontrol formulation. The temperature in all plastic houses was 12–30 °C during treatment days, detected by maximum and minimum thermometer in each plastic house.

2.4. Field trials in Jiang'su and Hu'bei provinces

According to the results in 2001, the best dose for controlling Phytophthora blight for each application method was determined. Field trials using the different methods with the best doses were conducted in Jiang'su and Hu'bei provinces in large plastic houses in 2002 and 2003, except for the laborious watering method. Each trial had both Ferment and water controls. The doses for the mixing, composting, and spraying treatments, which gave best control efficiency, were: 15, 7.5, and 15 L/ha of BF mixture, respectively. All treatment methods, i.e., mixing, composting, spraying, and two controls, were as described in this Section. All treatments were replicated four times and a completely randomized design was used. Biocontrol efficiency was assessed as the percentage reduction in disease relative to the water control.

2.5. Data analysis

Data were analyzed using Statistical Analysis Software (SAS Institute, Cary, NC, USA). One-way analysis of variance (ANOVA) was used. Means were separated using the least-significant difference test (LSD), $P = 0.05$.

3. Results

3.1. Greenhouse experiments

Mean biocontrol efficiency against Phytophthora blight and yield increase of the BF mixture reached 60.3 and 200%, respectively, while those for the BB11 and FH17 treatments were, respectively, 55.8 and 80.6%, and 37.1 and 50.0% (Table 1). The individual strain treatments had some biocontrol and yield-increase effects. The control and yield effects of the BF mixture treatment were superior to those for each individual strain treatment.

3.2. Application of different methods in the field

In field experiments at Huai'an in 2001, each method had its own best application dose. In the mixing treatment with rapeseed residue application approach, an application

Table 1
Biocontrol efficiency and yield increase produced by the BF mixture and individual biocontrol strains in greenhouse experiment

Treatment	Disease incidence (%)	Biocontrol efficiency (%)	Yield (kg)	Yield increase (%)
BB11	13.0c*	55.8b	32.5b	80.6b
FH17	18.5b	37.1c	27.0b	50.0b
BF mixture	7.29d	60.3a	54.0a	200a
Water	29.5a	0d	18.0c	0d
$LSD_{0.05}$	2.89	3.45	10.51	47.57

* Means followed by the same letter within a column were not significantly different ($P > 0.05$; LSD test). The biocontrol efficiency was calculated by disease index. Fruit production was determined by totaling three harvests from each plot, with all sizes picked and counted.

rate of 15 L/ha resulted in superior disease control and greater yield than any other application rate— reducing disease incidence by 87.9% and increasing yield by 60.1%, relative to water control (Table 2). In the composting, spraying, and watering methods, applications of 7.5, 15, and 22.5 L/ha BF mixture gave better disease control efficiency and yield increase, except for spraying, whose yield increase was slightly lower than that of 22.5 L/ha (Tables 3–5). The results also showed that the different application methods of the BF mixture led to different biocontrol efficiencies and yield increases. The average control efficiencies for mixing, composting, spraying, and watering were 87.9, 88.9, 88.2, and 85.7%, respectively, with their best doses of 15, 7.5, 15, and 22.5 L/ha.

Table 2
Effect of the BF mixture applied by mixing with rapeseed residue in a field experiment at Huai'an in 2001

Treatments	Dose (L/ha)	Disease incidence (%)	Control efficiencies (%)	Yields (kg/plot)	Yield increase (%)
Mixing	3	12.5c*	51.2c	90.2c	26.2c
	7.5	7.2c	71.9b	98.2b	37.3b
	15	3.1d	87.9a	114.5a	60.1a
	22.5	6.8c	73.4b	110.2a	54.1a
Water		25.6a		71.5d	
$LSD_{0.05}$		1.23	4.13	3.40	5.00

Mixing: the BF mixture (10^{10} cfu/ml), diluted with water (4000 L/ha), was sprayed onto rapeseed residue (2250 kg/ha), mixed thoroughly, and the residue scattered onto plots. The plots were ploughed separately, covered with plastic membranes, and plants were transplanted after 7 days. Water refers to water control. All treatments and control had four replicates.

* Means followed by the same letter within a column were not significantly different ($P > 0.05$; LSD test). The biocontrol efficiency was calculated by disease index. Fruit production was determined by totaling three harvests from each plot, with all sizes picked and counted.

Table 3
Effect of the BF mixture applied by composting in a field experiment at Huai'an in 2001

Treatments	Dose (ml)	Disease incidence (%)	Control efficiencies (%)	Yields (kg/plot)	Yield increase (%)
Composting	3	6.2b*	59.5c	100.2c	33.2c
	7.5	1.7d	88.9a	127.0a	68.9a
	15	3.9c	74.5b	119.4a	58.8a
	22.5	4.3c	71.9b	123.1a	63.7a
Ferment		4.2c	72.5b	112.9b	50.1b
Water		15.3a		75.2d	
$LSD_{0.05}$		0.92	5.08	6.28	8.94

Composting: the BF mixture (10^{10} cfu/ml), diluted with water (4000 L/ha), was mixed with rapeseed residue (2250 kg/ha) thoroughly, the residue piled up, and covered with black plastic film to increase temperature. The compost was mixed twice daily to avoid extremely high temperatures during the 3-day natural fermentation process. The compost's humidity was kept between 70 and 90% and temperature between 25 and 50 °C. The compost was scattered over the plot and ploughed in. The plants were then transplanted. Ferment: 135 kg/ha ferment was mixed with rapeseed residue (2250 kg/ha) and treated in the same way as the composting treatment. All treatments had four replicates.

* Means followed by the same letter within a column were not significantly different ($P > 0.05$; LSD test). The biocontrol efficiency was calculated by disease index. Fruit production was determined by totaling three harvests from each plot, with all sizes picked and counted.

Table 4
Effect of the BF mixture applied by spraying in a field experiment at Huai'an in 2001

Treatments	Dose (L/ha)	Disease incidence (%)	Control efficiencies (%)	Yields (kg/plot)	Yield increase (%)
Spraying	3	4.9b*	52.0c	88.8c	7.0c
	7.5	2.8c	72.5b	93.2b	12.3b
	15	1.2d	88.2a	100.7a	21.3a
	22.5	1.8cd	82.4ab	102.3a	23.3a
Water		10.2a		83d	
$LSD_{0.05}$		1.02	9.89	3.14	4.02

Spraying: the BF mixture (10^{10} cfu/ml), diluted with water (4000 L/ha), was sprayed onto plots equally. Rapeseed residue (2250 kg/ha) had been ploughed into the soil 7 days before transplanting.

* Means followed by the same letter within a column were not significantly different ($P > 0.05$; LSD test). The biocontrol efficiency was calculated by disease index. Fruit production was determined by totaling three harvests from each plot, with all sizes picked and counted.

Table 5
Effect of the BF mixture by watering in a field experiment at Huai'an in 2001

Treatment	Dose (L/ha)	Disease incidence (%)	Control efficiencies (%)	Yields (kg/plot)	Yield increase (%)
Watering	3	8.9b*	52.9d	87.4d	14.5d
	7.5	7.1c	62.4c	92.1c	20.7c
	15	4.2d	77.8b	101.3b	32.8b
	22.5	2.7e	85.7a	112.3a	47.2a
Water		18.9a		76.3e	
$LSD_{0.05}$		1.17	6.01	3.55	4.90

Watering: the BF mixture (10^{10} cfu/ml), diluted with water (4000 L/ha), was poured onto the pepper roots just after transplanting. The soil had been treated with rapeseed residue (2250 kg/ha) before transplanting.

* Means followed by the same letter within a column were not significantly different ($P > 0.05$; LSD test). The biocontrol efficiency was calculated by disease index. Fruit production was determined by totaling three harvests from each plot, with all sizes picked and counted.

The results of field trials in Jiang'su and Wu'han during 2002 and 2003 (Tables 6 and 7) were in conformity with the results of 2001. The total average control efficiencies and yield rates for the mixing, composting, and spraying methods, at their best doses in the same plastic houses in 2002 and 2003, were, respectively, 81.0 and 33.1%, 88.0 and 44.3%, and 79.1 and 29.1% (Table 8). There was a significant difference between composting and mixing or spraying in control efficiency and yield increase, while there was no significant difference between mixing and spraying (Table 8). Combining the results from 2001 to 2003 and considering its lowest application dose, it was concluded that the composting method was the best application method for the BF mixture against Phytophthora blight. Also the application of 125 L BF mixture/ha gave 81.0% total average control efficiency with the mixing method or 79.1% with the spraying method, which would be acceptable to farmers in controlling Phytophthora blight in Jiang'su and Hu'bei Provinces. Actually some farmers preferred the mixing or spraying methods more than the composting method, due

Table 6
Biocontrol efficiencies and yield increases for the different methods with best doses in same field at Huai'an in 2002 and 2003

Treatment	Disease incidence (%)		Control efficiencies (%)		Yields (kg/plot)		Yield increase (%)	
	2002	2003	2002	2003	2002	2003	2002	2003
Mixing	2.6bc*	2.8cd	85.0ab	83.2ab	110.1b	103.1b	45.4b	31.2b
Composting	2.1c	1.8d	87.9a	89.2a	118.9a	113.9a	57.1a	44.9a
Spraying	3.2bc	3.1c	81.5b	81.4b	107.3c	102.3bc	41.7c	30.2b
Ferment	4.5b	4.9b	74.0c	70.7c	100.7d	98.9c	33.0d	25.8c
Water	17.3a	16.7a			75.7e	78.6d		
$LSD_{0.05}$	1.50	1.09	11.95	6.33	3.62	3.62	4.38	4.07

Mixing: the BF mixture (15 L/ha, 10^{10} cfu/ml), diluted with water (4000 L/ha), was sprayed onto rapeseed residue (2250 kg/ha), mixed thoroughly, and the residue scattered onto plots. The plots were ploughed separately, covered with plastic membranes, and plants were transplanted after 7 days. Composting: the BF mixture (7.5 L/ha, 10^{10} cfu/ml), diluted with water (4000 L/ha), was mixed with rapeseed residue (2250 kg/ha) thoroughly, the residue piled up, and covered with black plastic film to increase temperature. The compost was mixed twice daily to avoid extremely high temperatures during the 3-day natural fermentation process. The compost's humidity was kept between 70 and 90% and temperature between 25 and 50 °C. The compost was scattered over the plot and ploughed in. The plants were then transplanted. Spraying: the BF mixture (15 L/ha, 10^{10} cfu/ml), diluted with water (4000 L/ha), was sprayed onto plots equally. Rapeseed residue (2250 kg/ha) had been ploughed into the soil 7 days before transplanting. Ferment: 135 kg/ha ferment was mixed with rapeseed residue (2250 kg/ha) and treated in the same way as the composting treatment. Water refers to water control. All treatments had 16 replicates in total.

* Means followed by the same letter within a column were not significantly different ($P > 0.05$; LSD test). The biocontrol efficiency was calculated by disease index. Fruit production was determined by totaling three harvests from each plot, with all sizes picked and counted.

Table 7
Biocontrol efficiencies and yield increases for the different methods with best doses in same field at Wu'han in 2002 and 2003

Treatment	Disease incidence (%)		Control efficiencies (%)		Yields (kg/plot)		Yield increase (%)	
	2002	2003	2002	2003	2002	2003	2002	2003
Mixing	2.4c*	2.3bc	78.9ab	76.5ab	104.3b	109.0b	26.3b	12.6b
Composting	1.1c	1.3c	90.4a	86.7a	115.1a	117.1a	38.8a	35.8a
Spraying	2.7bc	2.1bc	76.3b	78.6ab	99.0b	107.1b	19.4b	24.2b
Ferment	3.2b	3.2b	71.9c	67.3b	94.1c	100.2c	13.5c	16.2c
Water	11.4a	9.8a			82.9d	86.2d		
$LSD_{0.05}$	1.18	1.07	4.98	11.08	3.46	3.09	4.06	6.57

Mixing: the BF mixture (15 L/ha, 10^{10} cfu/ml), diluted with water (4000 L/ha), was sprayed onto rapeseed residue (2250 kg/ha), mixed thoroughly, and the residue scattered onto plots. The plots were ploughed separately, covered with plastic membranes, and plants were transplanted after 7 days. Composting: the BF mixture (7.5 L/ha, 10^{10} cfu/ml), diluted with water (4000 L/ha), was mixed with rapeseed residue (2250 kg/ha) thoroughly, the residue piled up, and covered with black plastic film to increase temperature. The compost was mixed twice daily to avoid extremely high temperatures during the 3-day natural fermentation process. The compost's humidity was kept between 70 and 90% and temperature between 25 and 50 °C. The compost was scattered over the plot and ploughed in. The plants were then transplanted. Spraying: the BF mixture (15 L/ha, 10^{10} cfu/ml), diluted with water (4000 L/ha), was sprayed onto plots equally. Rapeseed residue (2250 kg/ha) had been ploughed into the soil 7 days before transplanting. Ferment: 135 kg/ha ferment was mixed with rapeseed residue (2250 kg/ha) and treated in the same way as the composting treatment. Water refers to water control. All treatments had 16 replicates in total.

* Means followed by the same letter within a column were not significantly different ($P > 0.05$; LSD test). The biocontrol efficiency was calculated by disease index. Fruit production was determined by totaling three harvests from each plot, with all sizes picked and counted.

to the inconvenience of the latter, although 62.5 L/ha BF mixture in the composting method gave better average control results (88%). Compared with water control, application of the BF mixture resulted in significantly higher yields, regardless of application method. The differences in control efficiency among composting, mixing, and spraying methods were not completely significant, while the differences in yield increase were all significant (Tables 6 and 7). This indicated that the BF mixture either stimulated growth and fruiting of bell peppers or disease suppression improved plant growth and yield in the field. Table 8 also showed that the BF mixture had superior biocontrol efficiency than the ferment control. Therefore, the BF mixture has the potential of being a successful commercial formulation for the biocontrol of Phytophthora blight on bell peppers.

4. Discussion

In recent years, the incidence of Phytophthora blight has increased with continuous culture of some crops, resulting in significant loss in output and quality. At present, there is no single method of adequately controlling this disease on bell peppers. The purpose of this study was to develop one or more effective application methods for biocontrol agents.

In greenhouse experiments, mixing *Bacillus* preparations (BF mixture) showed superior biocontrol efficiency and growth promotion than each individual strains. This was consistent with the idea that bacterial mixtures provide a means of increasing the activity of a biocontrol treatment and improving performance (Pierson and Weller, 1994). Compared with the use of individual strains, mixtures of

Table 8
The total average biocontrol efficiencies and yield increase comparison of different method with their best doses in field experiment at Huai'an and Wu'han in 2002 and 2003

Treatment	Disease incidence (%)	Control efficiencies (%)	Yields (kg/plot)	Yield increase (%)
Mixing	2.5c*	81.0b	106.4b	31.1b
Composting	1.7c	88.0a	116.4a	44.3a
Spraying	2.8bc	79.1b	104.1b	29.1b
Ferment	3.8b	71.1c	97.3c	21.9c
Water	13.8a		80.7d	

Mixing: the BF mixture (15 L/ha, 10^{10} cfu/ml), diluted with water (4000 L/ha), was sprayed onto rapeseed residue (2250 kg/ha), mixed thoroughly, and the residue scattered onto plots. The plots were ploughed separately, covered with plastic membranes, and plants were transplanted after 7 days. Composting: the BF mixture (7.5 L/ha, 10^{10} cfu/ml), diluted with water (4000 L/ha), was mixed with rapeseed residue (2250 kg/ha) thoroughly, the residue piled up, and covered with black plastic film to increase temperature. The compost was mixed twice daily to avoid extremely high temperatures during the 3-day natural fermentation process. The compost's humidity was kept between 70 and 90% and temperature between 25 and 50 °C. The compost was scattered over the plot and ploughed in. The plants were then transplanted. Spraying: the BF mixture (15 L/ha, 10^{10} cfu/ml), diluted with water (4000 L/ha), was sprayed onto plots equally. Rapeseed residue (2250 kg/ha) had been ploughed into the soil 7 days before transplanting. Ferment: 135 kg/ha ferment was mixed with rapeseed residue (2250 kg/ha) and treated in the same way as the composting treatment. Water refers to water control. All treatments had 16 replicates in total.

* Means followed by the same letter within a column were not significantly different ($P > 0.05$; LSD test). The biocontrol efficiency was calculated by disease index. Fruit production was determined by totaling three harvests from each plot, with all sizes picked and counted.

several compatible strains may result in a more stable rhizosphere community, provide several mechanisms of biological control and even suppress a broader range of pathogens (Boer et al., 2003; Datnoff et al., 1995; Duffy and Weller, 1995; Duffy et al., 1996; Janisiewicz, 1988; Janisiewicz and Bors, 1995; Pierson and Weller, 1994; Raupach and Kloepper, 1998). A mix of different compatible antagonistic strains may function at different stages of the pathogen life cycle, rendering more effective protection (Boer et al., 2003; Mumpuni et al., 1998). Raupach and Kloepper (1998) used PGPR strains capable of eliciting induced systemic resistance (ISR) to control multiple cucumber pathogens. They concluded that PGPR mixtures provided synergistic activity against a broader range of pathogens on one host. On the other hand, greenhouse experiments in this study showed that the doses of PGPR mixtures yielding high levels of disease control were many times lower than those of individual strains, in agreement with other research and field experiment results (Russo, 2004).

Different application methods of the BF mixture lead to different biocontrol efficiencies and growth-promotion rates under the same fertilizer and management conditions in field trials. Total average biocontrol efficiencies and yield-promotion rates were much better when the BF mixture was applied by the composting method than by the mixing or spraying method. In this study, composting the BF mixture into rapeseed residue at least 7 days before field application produced the highest total average biocontrol efficiency (88%), which was 8.6% higher than the 'mixing with fertilizer' method (mixing the BF mixture with rapeseed residue and then immediately applying to the field) or spraying the BF mixture directly onto the field. Incidentally, the best application dose of composting was half the best dose for the mixing or spraying method, which may be commercially important in the popularization of this technique in the future. Data from other laboratories also showed good biocontrol efficiencies against soil-borne diseases with compost-amended container media (Chung and Hoitink, 1990; Spencer and Benson, 1982; Stephens and Stebbins, 1985). The composting method also resulted in the highest yield increase, which was at least one and half times those of the other two methods. Biocontrol strains might propagate much faster in compost than in the other two conditions, because the outer and inner temperature of the compost could reach 25 and 50 °C, respectively. This is beneficial to the growth and spore formation of *Bacillus* spp., while at the same time restraining the growth of indigenous nonspore-forming bacteria (Nakasaki et al., 1998). Spores are normally considered the main functional factor in using *Bacillus* spp. as biocontrol agents. However, the biocontrol strains, mixed with rapeseed residue and immediately scattered onto the field, propagated slowly or did not multiply (and even decreased) because of the lower comparative temperature and large number of indigenous bacteria in the soil (Nakasaki et al., 1992). After transplanting sweet peppers, the population of the biocontrol strains in the rhizosphere was much larger when applied as compost (unpublished data). So, as regards similar mechanisms, application as compost results in better biocontrol efficiency. Simultaneously with the rapid propagation of PGPR strains in compost, organic matter in rapeseed residue, such as fiber, proteins, and humic acids, are likely decomposed and easily absorbed by plants. This may not only increase yield but may also improve biocontrol efficiency, because organic-matter decomposition significantly influences the composition of bacterial species in the rhizosphere (Boehm et al., 1993).

Field experiments in 2001 showed that 15 L/ha was the best dose for the BF mixture when it was sprayed onto rapeseed residue that was immediately scattered onto fields, or sprayed onto the field directly—both methods used widely by farmers. Although they were not the best application methods, 15 L/ha BF mixture (10^{10} cfu/ml) was considered an acceptable dose in controlling Phytophthora blight on bell peppers, due to its superior biocontrol efficiency (85 or 81.5%). Chemical fungicides would be far more costly in achieving the same level of control, because the raw materials (potato and sucrose) used in the production of BF mixture preparation are comparatively cheap. The cost of mass-producing biocontrol agents is critical in determining the economic feasibility of any biocontrol strategy. Therefore, our results are especially encouraging for the economic feasibility of biologically controlling Phytophthora blight on bell peppers. The BF mixture has excellent prospects of being produced commercially, although its environmental impact needs study. Additional work is also necessary on the optimal application dose to gain acceptable control efficiency.

It is generally assumed that the biocontrol efficiency and yield are higher when the biocontrol agents are used in a high dosage, but in this experiment, the yield decreased after reaching 15 L/ha applied in the compost treatment. Probably too much introduced microbe could have a negative influence on the plant or the rhizosphere microbial community. We are doing some DGGE analysis for detecting the influence of biocontrol bacteria on the rhizosphere soil.

Acknowledgments

This research was supported by a Grant-in-Aid for science research from the Chinese 863 high-tech Program (2002AA244041), Jiangsu Province (JH02-084), and Sino-Germany Cooperation Projext on Agricultural Science and Technology (2004-Z17). The authors thank Dr Zhi-Ying Wang for his help in isolating and culturing *P. capsici*.

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