III. FITOSSANIDADE

BACILLUS SUBTILIS FOR THE CONTROL OF POWDERY MILDEW ON CUCUMBER AND ZUCCHINI SQUASH[1]

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ABSTRACT

Application of concentrated metabolites of *Bacillus subtilis* - CMBS - (5,000 µg/mL) one and 24 h before or after inoculation of *Sphaerotheca fuliginea* (3 x 10^4 conidia/mL) reduced the number of lesions on cucumber leaves by 90-99%. The average number of lesions on control plants was 16.7 per leaf. A wettable powder product formulated with cells (10%) and metabolites (10%) of *B. subtilis* (WPBS), and CMBS sprayed on cucumber plants (1,000 µg/mL and 10,000 µg/mL) twice a week totally controlled powdery mildew. In the control treatment, 18 days after the first spray, the percent leaf surface covered by lesions was 99.0 and 46.7%, on the cotyledonary and expanded leaves, respectively. In the control treatment, 30 days after the first spray, the percent leaf surface with lesions was 26.1%, while leaves sprayed with CMBS presented no lesions. The fresh weight per plant was 4.3 g in the control treatment; 12.2 g, and 10.2 g for plants sprayed with CMBS at the concentration of 1,000 and 10,000 µg/mL, respectively; and 9.7 g and 10.1 g for plants sprayed with WPBS 1,000 and 10,000 µg/mL, respectively. For zucchini squash, CMBS (5,000 µg/mL) sprayed every 2, 4, and 6 days showed reductions in lesioned leaf surface of 100.0, 98.3, and 94.7%, respectively.

Index terms: biological control, cucurbit, *Sphaerotheca fuliginea, Cucumis sativus, Cucurbita pepo.*

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RESUMO

CONTROLE DO OÍDIO DO PEPINO E DA ABÓBORA COM *BACILLUS SUBTILIS*

Aplicações de metabólitos concentrados produzidos por *Bacillus subtilis* - CMBS - (5.000 µg/mL), uma e 24 horas antes ou depois de inoculação de *Sphaerotheca fuliginea* (3 x 10^4 conídios/mL), reduziram o número de lesões nas folhas de pepino entre 90 e 99%. O número médio de lesões nas plantas pulverizadas com água foi de 16,7 lesões por folha. Um produto pó-molhável, formulado com células (10%) mais metabólitos (10%) de *Bacillus subtilis* (WPBS) e CMBS pulverizados em folhas de pepino (1.000 µg/mL e 10.000 µg/mL) duas vezes por semana controlaram totalmente o oídio das folhas, enquanto no tratamento controle, 18 dias após a primeira pulverização, a porcentagem de área foliar coberta com o patógeno foi de 99,0 e 46,7% nas folhas cotiledonares e folhas verdadeiras respectivamente. No tratamento controle, 30 dias após a primeira pulverização, a porcentagem de área foliar com lesões foi de 26,1%, enquanto as folhas pulverizadas com CMBS não apresentavam lesões. A massa de matéria fresca por planta foi de 4,3 g para as plantas pulverizadas com água; 12,2 g e 10,2 g para as pulverizadas com CMBS, nas concentrações de 1.000 e 10.000 µg/mL respectivamente, e 9,7 g e 10,1 g para as pulverizadas com WPBS nas concentrações de 1.000 e 10.000 µg/mL respectivamente. Para abóbora, o CMBS pulverizado a cada 2, 4 e 6 dias, na concentração de 5.000 µg/mL, reduziu a porcentagem de área foliar coberta com o patógeno em 100,0, 98,3 e 94,7% respectivamente.

Termos de indexação: controle biológico, cucurbitáceas, *Sphaerotheca fuliginea, Cucumis sativus, Cucurbita pepo.*

1. INTRODUCTION

Cucurbit powdery mildew, caused by *Sphaerotheca fuliginea* (Schlecht.) Pollacci, is an important cucurbit disease world-wide. It occurs on leaves, stems and fruits, but usually is more abundant on upper leaf surfaces. Control methods currently available to growers include the use of resistant cultivars (Boiteux et al., 1995), repeated applications of sulphur (Kimati et al., 1980), and fungicides (Kimati et al., 1986). The constant use of fungicides, however, can result in environmental contamination. Marco et al. (1994) described the suppression of powdery mildew on squash by applications of whitewash, clay and antitranspirant materials. Unfortunately, the protection level achieved with these materials was lower than with a fungicide (propiconazole).

Several authors (Garibaldi et al., 1994; Reuveni et al., 1995) verified that powdery mildew was significantly better controlled by a single spray of aqueous solutions containing various phosphates and potassium salts. These authors concluded that phosphates and potassium are appropriate leaf fertilizers, with a potential for disease control.

Biological control agents for cucurbit powdery mildew have been described. The most important ones are: *Tilletiopsis* spp. (Hijwegen, 1992; Urquhart et al., 1994), *Ampelomyces quisqualis* (Falk et al., 1995), *Cladosporium* sp. (Minuto et al., 1991), *Verticillium lecanii* (Verhaar & Hijwegen, 1993), and *Acremonium alternatum* (Malathrakis, 1985). As far as we know, no references are available on the biological control of *S. fuliginea* by *Bacillus subtilis,* which is effective in the control of other plant pathogenic fungi (Baker et al., 1983; Pusey & Wilson, 1984; Bettiol et al., 1992, 1994). This study aimed at evaluating the potential of concentrated metabolites (CMBS) and a product formulated with cells and metabolites (WPBS) of *B. subtilis* as to the control of powdery mildew on cucumber and zucchini squash.

2. MATERIAL AND METHODS

1. Experimental products used

Two experimental products with *B. subtilis* were tested for the control of *S. fuliginea*:

a) CMBS - concentrated metabolites of *B. subtilis*, isolate AP-3 (Bettiol & Kimati, 1990). *B. subtilis* was grown in Erlenmeyer flasks (1 L capacity) containing dextrose 1%, peptone 1%, yeast extract 0.5%, NaCl 0.3%, KH_2PO_4 0.1% and $MgSO_4$ 0.05%, placed on a rotary shaker at 150 rpm and 27 ± 2° C for 7 days. The fermentation medium was centrifuged at 13,200 g to remove bacteria cells. The antibiotics were precipitated from the supernatant by adjusting pH to 2.0 with HCl 1N. The resulting material was again centrifuged at 13,200 g, being the metabolites stored in bottles at 2° C.

b) WPBS - a wettable powder of *B. subtilis* was formulated with cells (60 g) and concentrated metabolites (60 g) of *B. subtilis*, clay (480 g - Sintermor Mineração Ltd.), surfactant (7.92 g - Surfon 950 PM - Oxiteno S.A.), and water (2.4 L). This mixture was crushed, sieved (0.210 mm) and dried (Spray-dryer set at 101 ± 1° C, at entry).

2. Effect of timing of CMBS application on severity of *S. fuliginea* attacks on cucumber

Cucumber plants (Marketmore Standard) were grown in polyethylene pots of 0.6 L containing a peat-based organic substrate until they reached the developmental stage of two expanded leaves. At this stage, plants were sprayed with CMBS (5,000 µg/mL) one and 24 h before or after inoculation with *S. fuliginea* (3×10^4 conidia/mL). Control plants were sprayed with water and, after 1 h, inoculated with the pathogen. The application was performed with a De Vilbiss # 15 hand sprayer until runoff. Experiment was set in a randomized block design with five replications per treatment with a replication consisting of one pot, containing five plants each.

After inoculation the plants were placed in a growth chamber (25 ± 2° C, 12 h light-6,000 lux). The plants were evaluated after 13 days by counting the number of lesions per leaf for the expanded leaves and by estimating the cotiledonary leaf surface covered by powdery mildew (Marco et al., 1994).

3. Effect of WPBS and CMBS concentrations on severity of *S. fuliginea* on cucumber

Cucumber plants (Mezzolungo-Marketer) seven days old, grown as described previously, were transferred to a growth chamber, with high *S. fuliginea* inoculum potential and sprayed twice a week with: a) water; b) CMBS (1,000 µg/mL); c) CMBS (10,000 µg/mL); d) WPBS (1,000 µg/mL); and e) WPBS (10,000 µg/mL). The plants were evaluated 18 and 30 days after the first spray by estimating the leaf surface covered by lesions. Fresh weight of the plants was evaluated at the end of the experiment. The experiment was set in a randomized block design, with five replications. Before the first disease evaluation, pots containing five plants had the number reduced to two plants for each pot.

4. Effect of spray frequency of CMBS on the effectiveness of *S. fuliginea* control on zucchini squash

Zucchini squash plants (IBIS NUOVO 423 F1), with three expanded leaves were grown in polyethylene pots of 2.0 L containing a peat based organic substrate, in a growth chamber (25 ± 2° C, 12 h light) with high *S. fuliginea* inoculum potential. These plants were sprayed with CMBS 5,000 µg/mL every 2, 4 and 6 days. Control plants were sprayed every two days with distilled water. The experiment was set in a randomized block design with five replications and two plants per pot.

The leaf surface with lesions was evaluated 8, 13 and 20 days after the first spray, and the fresh weight of leaves was evaluated 21 days after the first spray.

3. RESULTS AND DISCUSSION

On cucumber cotyledonary leaves CMBS sprayed one and 24 h before or after inoculation of *S. fuliginea* provided 100% of disease control, while the

percent leaf surface covered by lesions in the control was 5.7%. On fully expanded leaves the number of lesions on the control was 16.8 per leaf (Table 1), while the reduction of lesion number by CMBS sprayed 1 h before, 1 h after, and 24 h after inoculation were 99.4, 98.1, and 99.5%, respectively; CMBS sprayed 24 h before inoculation caused a reduction of 89.5%. Such an effect was possible due to the ectoparasitic habit of the pathogen similar results were obtained when *Cylindrocladium scoparium, Curvularia eragrostidis* and *Hemileia vastatrix* were inoculated before *B. subtilis* application, with no effective control observed (Bettiol et al., 1988; Bettiol & Varzea, 1992; Andrade et al., 1995). The result obtained with CMBS on *S. fuliginea*, however, indicates a broader range of timing for effective application with respect to the life cycle of the fungus.

CMBS and WPBS (1,000 and 10,000 µg/mL) sprayed on cucumber plants twice a week totally controlled the powdery mildew. In the control treatment the percent leaf surface covered by lesions 18 days after the first spray was 99.0 and 46.7% on the cotyledonary and expanded leaves, respectively (Table 2). Compared to the control plants, 30 days after the first spray the percent leaf surface with lesions and percent leaf surface with lesion per injuried leaf were 26.1 and 43.5%, respectively. The plants sprayed with CMBS showed no lesions. The fresh weight per plant was 4.3 g in the control plants, 12.2 g and 10.2 g for the plants sprayed with CMBS 1,000 and 10,000 µg/mL, respectively; 9.7 and 10.1 g for the plants sprayed with WPBS 1,000 and 10,000 µg/mL, respectively (Table 2). As no disease occurred after CMBS and WPBS application, no statistical analyses were performed on the disease variables of this experiment.

As far as plant fresh weight is concerned, treatments differed significantly ($P < 0.05$) from the control, and both CMBS and WPBS at 1,000 µg/mL and spray twice a week effectively increased plant weight.

When sprayed with CMBS every 2, 4 and 6 days zucchini squash showed reductions in leaf surface covered by lesions of 100.0, 98.3, and 95.5%, respectively. Considering the leaf surface with lesions per injuried leaf the values were 100.0, 96.9, and 91.9% for plants sprayed every 2, 4, and 6 days, respectively. The fresh weight for the control plot was 6.4 g/plant while for the plants sprayed every 2, 4, and 6 days were 13.5, 12.8, and 15.2 g/plant, respectively (Table 3).

Table 1. Effect of timing of application of concentrated metabolites of *Bacillus subtilis* (CMBS - 5,000 µg/mL), sprayed with hand sprayer until runoff, on the severity of *Sphaerotheca fuliginea* (3 x 10^4 spores/mL) on cucumber

Application of CMBS	Cotiledonary leaf surface with lesions	Expanded leaves	
		Number of lesions	Reduction of disease
	%		%
Water (control)	5.7	16.8a	–
1 h before pathogen inoculation	0.0	0.1b	99.4
1 h after pathogen inoculation	0.0	0.3b	98.1
24 h before pathogen inoculation	0.0	1.8b	89.5
24 h after pathogen inoculation	0.0	0.1b	99.5

Values followed by different letters are significantly different at the P = 0.05 level according to the Tukey test.

Table 2. Effect of concentrated metabolites of *Bacillus subtilis* (CMBS), and a wettable powder contained concentrated metabolites and cells of *B. subtilis* (WPBS) on severity of *Sphaerotheca fuliginea* on cucumber, and fresh weight of the plants

Treatment	Cotiledonary leaf surface with lesions	Expanded leaves: Leaf surface covered by lesions/injuried leaf (days after first spray)		Fresh weight/plant
		18	30	
µg/mL	%			g
Water (control)	99.0	46.7	43.5	4.26 b
CMBS 1,000	0.0	0.0	0.0	12.16 a
CMBS 10,000	0.0	0.0	0.0	10.20 a
WPBS 1,000	0.0	0.0	0.0	9.68 a
WPBS 10,000	0.0	0.0	0.0	10.06 a

Values followed by different letters are significantly different at the P = 0.05 level according to the Tukey test.

Table 3. Effect of spray frequency of concentrated metabolites of *Bacillus subtilis* (CMBS - 5,000 µg/mL) on effectiveness of *Sphaerotheca fuliginea* control on zucchini squash, evaluated 20 days after the first spray

Treatment	Leaf surface covered by lesions		Leaf surface covered by lesions/injuried leaf		Fresh weight/plant
	Reduction of disease				
	%				g
Water (control)	39.9 a	–	49.8 a	–	6.4 b
CMBS sprayed every 2 days	0.0 b	100.0	0.0 b	100.0	13.5 a
CMBS sprayed every 4 days	0.7 b	98.3	1.5 b	96.9	12.8 a
CMBS sprayed every 6 days	1.8 b	95.5	4.0 b	91.9	15.2 a

Values following by different letters are significantly different at the P = 0.05 level according to the Tukey test.

The results obtained indicated the efficacy of both products, CMBS and WPBS of *B. subtilis*, in controlling *S. fuliginea* on cucumber and zucchini squash. Further studies are needed to characterize the mechanism of action of such products and the role of antibiotic metabolites produced by *B. subtilis*, which could be the major responsible for the biocontrol achieved.

The efficacy of CMBS and WPBS for controlling powdery mildew (*S. fuliginea*) on cucumber and zucchini squash has been found to be similar to those observed by Bettiol et al. (1992, 1994) for *Uromyces appendiculatus* var. *appendiculatus* and *H. vastatrix*, and by Lazzaretti & Bettiol[4] for controlling several pathogens associated with rice, wheat, bush-bean and soybean seeds. Such efficacy corroborates the recognized capability of *B. subtilis*' metabolites for inhibiting many phytopathogenic fungi (Baker et al., 1983; McKeen et al., 1986; Pusey, 1989; Thirumalachar & O'Brien, 1977). These results indicate that, given the high-performance of *B. subtilis* metabolites against the studied disease on zucchini squash and cucumber, the CMBS and WPBS metabolite-compounds may contribute for the control of other pathogens associated to these crops.

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