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Effects of Chemical Inducers and *Paenibacillus* on Tomato Growth Promotion and Control of Bacterial Wilt

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ABSTRACT

Tomato (*Solanum lycopersicum* L.) is one of the world's most widely cultivated vegetable crops. Among the tomato-inflicting diseases, wilt caused by *Ralstonia solanacearum* (Smith) is a major yield-limiting factor. Management of the disease has been hampered by use of ineffective pesticides and lack of resistant varieties. This study aimed at using two antagonistic bacteria and three chemical inducers to inhibit the disease. The dual-culture technique was used to test the in vitro inhibition of *R. solanacearum* by two antagonistic bacteria (*Paenibacillus polymyxa* MB02-1007 and *Paenibacillus macerans* MB02-992) and three chemical inducers (sodium benzoate, ascorbic acid and isonicotinic acid). The effects of the two antagonistic bacteria and three chemical inducers on control of *Ralstonia* wilt and promotion of tomato growth were evaluated in pots in a randomized block design under greenhouse conditions. The antagonistic bacteria significantly improved seed germination and seedling vigour of tomato plants. Disease incidence and the population of *Ralstonia solanacearum* in tomato plants were considerably reduced by the two antagonistic bacteria and the three chemical inducers, singly or in combination, compared to the control. In particular, the combination of antagonistic bacteria with isonicotinic acid at 3 mg mL⁻¹ increased height, fresh and dry weight of tomato plants by more than 89%, while the combination of antagonistic bacteria with sodium benzoate at 40 mg mL⁻¹ or ascorbic acid at 8 mg mL⁻¹ or isonicotinic acid at 3 mg mL⁻¹ inhibited tomato bacterial wilt by more than 72% compared to the control. Overall, this study revealed that chemical inducers, in combination with antagonistic bacteria, have a powerful effect on tomato growth promotion and control of tomato bacterial wilt.

Key words: *Paenibacillus*, ascorbic acid, isonicotinic acid, sodium benzoate, *Solanum lycopersicum*

INTRODUCTION

Ralstonia solanacearum, the causal agent of bacterial wilt disease, is a major obstacle in the production of solanaceous plants in both tropical and temperate regions (Algam *et al.*, 2004, 2005, Soad *et al.*, 2006). The lack of effective pesticides and resistant varieties call for the development of alternative strategies for the management of this disease. Biological control is one of the promising approaches to reduce disease incidence and hence yield losses caused by this disease (Guo *et al.*, 2004; Park *et al.*, 2007; Thanh *et al.*, 2009; Xue *et al.*, 2009). *Paenibacillus macerans* MB02-992 and *Paenibacillus polymyxa* MB02-1007 reduced *Pythium* damping-off in cucumber

(Li *et al.*, 2007) and increased root fresh weight of mycorrhizal cucumber (Li *et al.*, 2008b). In particular, they inhibited in vitro growth of *R. solanacearum*, while *P. polymyxa* MB02-1007 considerably increased resistance of tomato plants to bacterial wilt when applied as a soil drench or seed treatment (Algam *et al.*, 2010).

Sodium benzoate, ascorbic acid and isonicotinic acid have also been reported to induce resistance in plants to a number of plant pathogens (Kataria *et al.*, 1997; Oostendorp *et al.*, 2001; Algam *et al.*, 2010). In addition, several recent studies have indicated that highly effective biological control of soil-borne plant pathogens could be realized with the combined application of chemical inducers and microbial biocontrol agents (Anith *et al.*, 2004; Siddiqui, 2004; Siddiqui and Akhtar, 2008; Amini, 2009; Rahman *et al.*, 2009). However, there is little information concerning the effects of these chemical inducers, alone or in combination with antagonistic bacteria, on plant growth and the incidence of bacterial wilt in tomato.

The objective of this study was to examine the effect of three chemical inducers and two antagonistic bacteria, alone or in combination, on the control of bacterial wilt and promotion of tomato growth.

MATERIALS AND METHODS

Strains used in this study: The virulent strain *R. solanacearum* Rs-f.91 (race 1/biovar 3/phylotype 1) was identified by fatty acid methyl ester (FAME) analysis using the Sherlock system (MIDI, USA). The pathogenicity of *R. solanacearum* Rs-f.91 on tomato plants was confirmed in preliminary experiments and the strain was deposited in the Culture Collection of Fujian Academy of Agricultural Sciences and at the Institute of Biotechnology, Zhejiang University, China. The strain was routinely cultured on Casamino Acid Peptone Glucose (CPG) agar or on Tetrazolium Chloride (TTC) agar at 28°C for 48 h (Algam *et al.*, 2010) or temporarily stored in sterile distilled water at room conditions.

Seven strains of *P. polymyxa* (MB02-226, MB02-376, MB02-428, MB02-1007, MB02-1172, MB02-1202 and MB02-1265) and nine strains of *P. macerans* (MB02-167, MB02-429, MB02-513, MB02-523, MB02-454, MB02-727, MB02-992, MB02-1125 and MB02-1180) were kindly provided by the Department of Integrated Pest Management, Faculty of Agricultural Science, University of Aarhus, Denmark. We reported earlier that in vitro growth of *R. solanacearum* was inhibited by *P. polymyxa* MB02-1007 and *P. macerans* MB02-992, but was not affected by all other strains (Algam *et al.*, 2010). Accordingly, these two strains were selected for use in the present study and were cultured on Nutrient Agar (NA) supplemented with 5% sucrose (Li *et al.*, 2008a) or stored in 20% aqueous glycerol at -70°C for further studies.

Chemical inducers: Sodium benzoate (SB), ascorbic acid (AsA) and isonicotinic acid (INA), purchased from Sigma-Aldrich (St. Louis, MO, USA), were dissolved in deionized water in order to obtain two different concentrations of each (SB at 40 and 80 mg mL⁻¹, As A at 4 and 8 mg mL⁻¹, INA at 3 and 5 mg mL⁻¹). These concentrations were chosen, with minor modifications, based on their performance as reported by El-Mougy *et al.* (2004) and El-Gamal *et al.* (2007).

Effects of chemical inducers and antagonistic bacteria: Cells of the antagonistic bacteria from plate culture (48 h old) were washed twice in sterile distilled water and resuspended in sterile distilled water. Bacterial suspension was adjusted to an optical density of OD₆₀₀ = 1 (10⁸ CFU mL⁻¹) using a spectrophotometer. Two µL of bacterial suspension were mixed with 15 mL of NA at 50°C and were then poured into petri plates. After solidification, three sterile filter paper discs were

placed at equidistance in a medium seeded with the antagonistic bacteria. Five μL of each concentration of the chemical inducers were added to a filter paper disc, while 5 μL of sterile distilled water were added to the control filter paper disc. Triplicate plates were used in each treatment. The plates were incubated at $28\pm 2^\circ\text{C}$ for 48 h and observed for the appearance of inhibition zones. Absence of an inhibition zone around the disc indicated that the growth of antagonistic bacteria was unaffected by the chemical inducers, while the presence of an inhibition zone indicated inhibition (Fukui *et al.*, 1994).

***In vitro* inhibition of *R. solanacearum*:** The effects of chemical inducers or antagonistic bacteria, alone or in combination, on the *in vitro* growth of *R. solanacearum* were determined by the dual culture technique (Li *et al.*, 2008c; Algam *et al.*, 2010). *R. solanacearum* was grown overnight in CPG broth at 30°C and the suspension was adjusted to 1×10^8 CFU mL^{-1} using a spectrophotometer. One milliliter of the suspension was then added to 15 mL of molten CPG agar at 50°C in a petri plate and the medium was allowed to solidify. Sterilized filter paper discs of 3 mm diameter were spotted with 6 μL each of the three chemical inducers at the two above concentrations, 2 μL of antagonistic bacterial suspension at 10^8 CFU mL^{-1} , or a combination of both and were then placed onto petri plates. Each investigation was carried out in triplicates. The plates were incubated at room temperature ($28\pm 2^\circ\text{C}$) for 48 h and the diameter of the inhibition zone was measured in mm.

Seed germination and seedling vigour: The chemical inducers at two different concentrations (SB at 40 and 80 mg mL^{-1} ; As A at 4 and 8 mg mL^{-1} ; INA at 3 and 5 mg mL^{-1}), the antagonistic bacteria, or the combination of both, were tested on tomato seed emergence. The antagonistic bacteria, grown on NA for 48 h, were suspended in sterile distilled water and the suspension was adjusted to approximately 1×10^8 CFU mL^{-1} . 'Hezou' tomato seeds, obtained from the Horticulture Department, Zhejiang University, China, were surface sterilized with 2% sodium hypochlorite for 5 min and washed several times with sterilized water (Algam *et al.*, 2010). One gram of seeds was soaked in 10 mL of the bacterial suspension, chemical inducers, or their combination for 2 h and left to dry overnight in a flow cabinet, placed onto sterile filter paper moistened with sterile distilled water in petri plates (about 20 seeds per plate) and incubated at 28°C for 7 d. Control plates were arranged in a similar way, except that seeds were treated with 10 mL of either sterile distilled water or the commercial bactericide ShiLeShi (Chemical name: Fludioxonil). Each treatment had three replicates. Seed germination was measured by counting the number of fully germinated seeds per plate (Nejad and Johnson, 2000). The viability of seedlings was determined by calculating the vigour index (VI) of the seeds, using the formula of Abdul-Baki and Anderson (1973): (mean shoot length+mean root length) \times percentage of germination.

Greenhouse assays control of tomato wilt and promotion of tomato growth: 'Hezou' tomato seeds were surface sterilized as described above. Pre-germinated seeds were sown in pots of 20 cm diameter and 20 cm height containing unsterilized natural vegetable soil (clay-sandy soil, pH 6.6). The experiment was conducted during Jan.-June 2009 in Hangzhou, China. Plants were maintained in a temperature-controlled glasshouse ($28\pm 2^\circ\text{C}$) with Osram daylight lamps providing supplementary light for a 12 h photoperiod, with 70-80% humidity. Each treatment consisted of four pots (replicates) with 5 plants per pot. The pots were arranged in a randomized block design. The experiment was conducted twice.

Seeds were treated with 10 mL of the chemical inducers at the above-mentioned two concentrations or with 10 mL of antagonistic bacterial suspension or with 5 mL chemical inducers

and 5 mL antagonistic bacteria for 2 h and were then left to dry overnight in a flow cabinet. Seeds were then placed onto sterile filter paper moistened with sterile distilled water and incubated at room temperature for 7 d. Seeds in the control treatment were immersed in 10 mL of either sterile distilled water or the commercial bactericide ShiLeShi.

Inoculum of the pathogen was prepared by incubating *R. solanacearum* Rs-f.91 overnight in CPG broth at 30°C on a rotary shaker (130 rpm per min) and harvested by centrifugation for 5 min at 10,000 rpm. The cell pellets were diluted in sterile distilled water and the bacterial concentration was adjusted to 1 (10^8 CFU mL⁻¹). Three weeks after sowing, tomato plants were inoculated with 30 mL of *R. solanacearum* by cutting roots with a sterile scissors and then drenching the cut roots with the *R. solanacearum* suspension, while plants in the control treatment received an equal amount of sterile water. After inoculation, tomato plants were covered with plastic bags for 24 h to maintain high humidity as described by Algam *et al.* (2010).

Four weeks after inoculation, stem tissues were collected from one plant in each pot to assess *R. solanacearum* counts in the plants. One gram of the lower stem internodes (15 to 20 cm above the soil) of each treatment was washed with tap water, surface sterilized with 3% sodium hypochloride for 5 min and washed several times with sterile water. Stem tissues were homogenized in a sterile mortar and pestle with 10 mL of 0.1 M potassium phosphate buffer (pH 7.0). Stem homogenates were serially diluted (10^{-1} to 10^{-9}) with 0.1 M potassium phosphate buffer. Then 100 µL of each dilution were transferred onto TTC medium and spread by a glass rod. Plates were incubated at 28°C for 48 h and the number of bacterial colonies was determined.

The incidence of tomato wilt was recorded periodically up to 8 weeks using a scale of 0-4 as described by Kempe and Sequeira (1983). Wilt incidence was calculated by the method of Algam *et al.* (2010). In addition, the heights of seedlings from all treatments were measured and plants were then removed from the pot, washed with distilled water, blotted with tissue paper and fresh weights were determined. Seedlings were then dried at 60°C for 72 h to determine their dry weights.

Statistical analysis: Data from the repeated experiment were combined using the General linear model analysis (GLM) procedure of SAS (SAS Institute, Cary, NC). Level of significance of the main factors and their interactions were calculated by two-way analyses of variance. Treatment means were compared using the LSD test.

RESULTS AND DISCUSSION

As the chemical inducers sodium benzoate (SB), ascorbic acid (AsA) and isonicotinic acid (INA) did not produce any inhibitory effect on the *in vitro* growth of the two *Paenibacillus* strains (*P. polymyxa* MB02-1007 and *P. macerans* MB02-992), both the chemical inducers and *Paenibacillus* strains were used in combination to examine their effect on suppression of tomato wilt both *in vitro* and *in vivo* experiments.

***In vitro* inhibition of *R. solanacearum*:** The *in vitro* growth of *R. solanacearum* was significantly inhibited by the two *Paenibacillus* strains, by the three chemical inducers and by their combination. The diameters of the inhibition zones produced by *P. polymyxa* MB02-1007 and *P. macerans* MB02-992 against *R. solanacearum* Rs-f.91 were 13.0 mm and 4.0 mm, respectively, while those produced by the three chemical inducers ranged from 4.0 to 7.0 mm. Diameters of inhibition zones resulting from the combined effect of the chemical inducers and *Paenibacillus* strains ranged from 15.0 to 17.0 mm.

Seed germination: The germination of tomato seeds treated with *P. macerans* MB02-992 or *P. Polymyxa* MB02-1007 was increased by 9.64 and 9.88%, respectively which was significant compared to the control (Table 2, 3). Likewise, the percentage germination was significantly ($p = 0.05$) increased by the three chemical inducers (Table 2, 3). As A at 8 mg mL^{-1} caused the most increase, followed by INA at 3 mg mL^{-1} , with an increase in germination percentage by 23.23% and 20.61%, respectively (Table 2, 3). However, the promotion effect of the combination of chemical inducers and antagonistic bacteria on germination was generally greater than that of either the chemical inducers or antagonistic bacteria alone. The highest germination of tomato seeds was obtained by the combination of INA at 3 mg mL^{-1} with *P. polymyxa* MB02-1007 which significantly ($p = 0.05$) increased the percentage germination by 32.3% compared to the control (Table 2, 3).

Seedling vigour: *P. macerans* MB02-992 and *P. polymyxa* MB02-1007 significantly increased seedling vigour by 16.15% and 16.88%, respectively (Table 2, 3). Seedling vigour was also significantly ($p = 0.05$) increased by As A at 4 and 8 mg mL^{-1} , INA at 3 and 5 mg mL^{-1} and SB at 40 mg mL^{-1} but was unaffected by SB at 80 mg mL^{-1} (Tables 2, 3). The significant increase in seedling vigour by As A at 8 mg mL^{-1} was followed by INA at 3 mg mL^{-1} which increased seedling vigour by 74.23 and 70.71%, respectively (Table 2, 3). However, the significant promotion by the combination of the chemical inducers and antagonistic bacteria in seedling vigour was greater than that of either the chemical inducers or antagonistic bacteria alone. The highest seedling vigour was obtained by the combination of INA at 3 mg mL^{-1} with *P. polymyxa* MB02-1007 which significantly increased seedling vigour by 93.98% compared to the control (Table 2, 3).

Growth promotion of tomato plants and control of tomato wilt: Height, fresh and dry weight of tomato plants were significantly ($p = 0.05$) increased by the three chemical inducers, antagonistic bacteria and their combination (Table 4, 5). The growth promotion by the combination of the chemical inducers and antagonistic bacteria in plant biomass was in general greater than that of either the chemical inducers or antagonistic bacteria alone. However, the effect of the combination of chemical inducers and antagonistic bacteria was significant for plant height and fresh weight, but not for dry weight (Table 1). *P. polymyxa* MB02-1007, in combination with INA at 3 mg mL^{-1} , significantly increased height, fresh and dry weight of tomato plants by 101.1, 108.3

Table 1: Probability (P) values for all measured parameters from two-way analyses of variance for chemical inducers and antagonistic bacteria as the main factors and their interaction

Parameters	Bacteria (B)	Chemical inducers (C)	B×C
Plant promotion			
Germination	**	0.14	***
MSL	0.07	0.07	0.10
MRL	0.06	0.07	0.21
Vigour index	*	*	***
Height	*	*	***
Fresh weight	*	*	***
Dry weight	*	*	0.18
Biocontrol			
Wilt incidence	**	*	***
Rs population	0.07	0.18	**

* = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$; MSL = Mean Shoot Length; MRL = Mean Root Length; Rs = *Ralstonia solanacearum*

Table 2: Effect of *Paenibacillus macerans*, chemical inducers, or their combination on seed germination and seedling vigour of tomato plants

Treatments	Germination %	MSL (cm)	MRL (cm)	Vigour index
Water	74.6 ^a	2.0 ^a	5.0 ^{cd}	515.2 ^a
Fludioxonil	76.3 ^a	1.9 ^a	4.9 ^c	516.8 ^a
Pm 992	81.8 ^c	2.9 ^d	4.4 ^{ab}	598.4 ^b
SB 40	82.7 ^c	2.7 ^{bc}	4.7 ^b	605.1 ^c
SB 80	77.3 ^{ab}	2.3 ^b	4.3 ^a	515.8 ^a
SB 40+Pm 992	85.7 ^d	3.7 ^{ef}	5.6 ^e	797.5 ^e
SB 80+Pm 992	82.2 ^c	2.9 ^d	5.1 ^d	656.9 ^{cd}
As A 4	83.2 ^d	3.2 ^e	4.8 ^{bc}	663.3 ^{cd}
As A 8	91.9 ^e	3.8 ^f	6.0 ^{ef}	897.7 ^e
As A 4+Pm 992	86.9 ^d	3.9 ^{ef}	5.7 ^e	836.8 ^{cd}
As A 8+Pm 992	96.8 ^f	3.7 ^{ef}	6.3 ^f	966.6 ^h
INA 3	89.9 ^e	3.9 ^{ef}	5.9 ^{ef}	879.5 ^e
INA 5	85.8 ^d	3.8 ^f	5.6 ^e	807.1 ^e
INA 3+Pm 992	93.7 ^{ef}	3.9 ^{ef}	6.5 ^{ef}	972.2 ^f
INA 5+Pm 992	85.2 ^d	3.9 ^{ef}	6.2 ^f	854.9 ^f

Data from the repeated experiment were pooled and subjected to analysis of variance. Means in a column followed by the same letter are not significantly different according to LSD test ($p = 0.05$). Pm = *Paenibacillus macerans*; MSL = Mean Shoot Length; MRL = Mean Root Length; SB 40 and 80 = Sodium Benzoate at 40 and 80 mg mL⁻¹; As A 4 and 8 = Ascorbic Acid at 4 and 8 mg mL⁻¹; INA 3 and 5 = Isonicotinic Acid at 3 and 5 mg mL⁻¹; Rs = *Ralstonia solanacearum*

Table 3: Effect of *Paenibacillus polymyxa*, chemical inducers or their combination on seed germination and seedling vigour of tomato plants

Treatments	Germination %	MSL(cm)	MRL (cm)	Vigour index
Water	74.6 ^a	2.0 ^a	5.0 ^{cd}	515.2 ^a
Fludioxonil	76.3 ^a	1.9 ^a	4.9 ^c	516.8 ^a
Pp 1007	81.9 ^b	2.9 ^d	4.5 ^{ab}	602.2 ^b
SB 40	82.7 ^b	2.7 ^{bc}	4.7 ^b	605.1 ^b
SB 80	77.3 ^a	2.3 ^b	4.3 ^a	515.8 ^a
SB 40+Pp 1007	87.2 ^d	3.7 ^{ef}	5.9 ^f	837.4 ^f
SB 80+Pp 1007	82.3 ^b	3.0 ^d	5.4 ^e	688.3 ^c
As A 4	83.2 ^{bc}	3.2 ^{de}	4.9 ^c	673.3 ^c
As A 8	91.9 ^e	3.8 ^{ef}	6.0 ^f	897.7 ^e
As A 4+Pp 1007	89.5 ^{de}	4.0 ^{gh}	4.9 ^c	792.5 ^{de}
As A 8+Pp 1007	97.9 ^f	3.8 ^{ef}	6.3 ^f	987.7 ^f
INA 3	89.9 ^{de}	3.9 ^{ef}	5.9 ^f	879.5 ^e
INA 5	80.8 ^b	3.6 ^{ef}	5.6 ^{ef}	739.9 ^d
INA 3+Pp 1007	98.7 ^f	3.7 ^{ef}	6.4 ^f	999.4 ^f
INA 5+Pp 1007	86.7 ^d	4.0 ^{gh}	6.5 ^f	903.0 ^d

Data from the repeated experiment were pooled and subjected to analysis of variance. Means in a column followed by the same letter are not significantly different according to LSD test ($p = 0.05$). Pp = *Paenibacillus polymyxa*; MSL = Mean Shoot Length; MRL = Mean Root Length; SB 40 and 80 = Sodium Benzoate at 40 and 80 mg mL⁻¹; As A 4 and 8 = Ascorbic Acid at 4 and 8 mg mL⁻¹; INA 3 and 5 = Isonicotinic Acid at 3 and 5 mg mL⁻¹; Rs = *Ralstonia solanacearum*

and 277.8%, respectively, compared to the pathogen-infected plants (Table 4, 5). Similar results were obtained using As A at 8 mg mL⁻¹ in combination with *P. polymyxa* MB02-1007 which increased height, fresh and dry weight by 84.65, 105.54 and 269.59%, respectively, relative to the pathogen-infected plants. *P. macerans* MB02-992, in combination with INA at 3 mg mL⁻¹, significantly increased height, fresh and dry weight of tomato plants by 89.84, 98.81 and 223.20%,

Table 4: Effect of *Paenibacillus macerans*, chemical inducers or their combination on fresh and dry weight and height of tomato plants inoculated with *Ralstonia solanacearum* Rs-f.91 under greenhouse conditions

Treatments	Height (cm)	Fresh weight (g)	Dry weight (g)
None	20.9 ^{bc}	18.3 ^b	2.9 ^{bc}
Rs control	16.7 ^a	16.8 ^a	1.9 ^a
Fludioxonil	18.8 ^b	17.5 ^{ab}	2.7 ^b
Pm 992	23.4 ^c	21.9 ^c	3.8 ^d
SB 40	23.6 ^c	25.8 ^d	3.6 ^d
SB 80	21.9 ^{bc}	25.2 ^d	3.3 ^d
SB 40+Pm 992	25.9 ^c	29.0 ^e	4.6 ^e
SB 80+Pm 992	23.9 ^c	26.8 ^e	4.1 ^e
As A 4	24.0 ^c	26.6 ^e	3.7 ^d
As A 8	24.9 ^{de}	30.1 ^f	5.5 ^f
As A 4+Pm 992	25.8 ^e	27.9 ^f	4.7 ^e
As A 8+Pm 992	27.4 ^f	32.6 ^g	5.9 ^{fg}
INA 3	29.8 ^h	30.8 ^f	6.0 ^{fg}
INA 5	25.2 ^e	29.7 ^h	5.8 ^f
INA 3+Pm 992	31.8 ⁱ	33.4 ^k	6.3 ^h
INA 5+Pm 992	26.7 ^f	31.2 ^j	5.8 ^f

Data from the repeated experiment were pooled and subjected to analysis of variance. Means in a column followed by the same letter are not significantly different according to LSD test (P = 0.05). Pm = *Paenibacillus macerans*; SB 40 and 80 = Sodium Benzoate at 40 and 80 mg mL⁻¹; As A 4 and 8 = Ascorbic Acid at 4 and 8 mg mL⁻¹; INA 3 and 5 = Isonicotinic Acid at 3 and 5 mg mL⁻¹; Rs = *Ralstonia solanacearum*

Table 5: Effect of *Paenibacillus polymyxa*, chemical inducers or their combination on fresh and dry weight and height of tomato plants inoculated with *Ralstonia solanacearum* Rs-f.91 under greenhouse conditions

Treatments	Height (cm)	Fresh weight (g)	Dry weight (g)
None	20.9 ^{bc}	18.3 ^b	2.9 ^{bc}
Rs control	16.7 ^a	16.8 ^a	1.9 ^a
Fludioxonil	18.8 ^b	17.5 ^{ab}	2.7 ^b
Pp 1007	23.9 ^d	22.7 ^c	3.9 ^e
SB 40	23.6 ^d	25.8 ^d	3.6 ^{de}
SB 80	21.9 ^{bc}	25.2 ^d	3.3 ^d
SB 40+Pp 1007	28.4 ^f	29.8 ^f	5.2 ^g
SB 80+Pp 1007	25.8 ^f	27.0 ^e	3.8 ^e
As A 4	24.0 ^d	26.6 ^e	3.7 ^{de}
As A 8	24.9 ^e	30.1 ^e	5.5 ^f
As A 4+Pp 1007	28.7 ^e	29.4 ^f	5.3 ^f
As A 8+Pp 1007	30.9 ^f	34.5 ^g	7.2 ⁱ
INA 3	29.8 ^h	30.8 ^f	6.0 ^{fg}
INA 5	25.2 ^e	29.7 ^g	5.8 ^{fg}
INA 3+Pp 1007	33.7 ⁱ	35.0 ^g	7.3 ⁱ
INA 5+Pp 1007	29.7 ^h	32.3 ^h	6.7 ^h

Data from the repeated experiment were pooled and subjected to analysis of variance. Means in a column followed by the same letter are not significantly different according to LSD test (P = 0.05). Pp = *Paenibacillus polymyxa*; SB 40 and 80 = Sodium Benzoate at 40 and 80 mg mL⁻¹; As A 4 and 8 = Ascorbic Acid at 4 and 8 mg mL⁻¹; INA 3 and 5 = Isonicotinic Acid at 3 and 5 mg mL⁻¹; Rs = *Ralstonia solanacearum*

respectively, while its combination with As A at 8 mg mL⁻¹ significantly increased height, fresh and dry weight of tomato plants by 63.92, 94.04 and 206.19%, respectively, compared to the pathogen-infected plants (Table 4, 5). Algam *et al.* (2010) found that the antagonistic bacteria *P. polymyxa*

MB02-1007 and *P. macerans* MB02-992 increased seed germination and seedling vigour along with improved fresh and dry weight of tomato plants compared to the control under greenhouse conditions. Kone *et al.* (2009) reported that squash fresh weight and height in most of the INA and acibenzolar-S-methyl treatments were significantly increased compared with the control treatment. In addition, these findings can be related to some earlier studies in which it was observed that exogenous application of As A promoted growth in wheat (Al-Hakimi, 2001; Hamada and Al-Hakimi, 2001) and tomato (Shalata and Neumann, 2001). Furthermore, As A-induced growth promotion may have been due to accelerated cell division and/or cell enlargement as postulated by Khan *et al.* (2006) and stomatal regulation and floral induction as suggested by Athar *et al.* (2009).

The interaction between chemical inducers and antagonistic bacteria was significant in reducing the incidence of wilt (Table 1). Bacterial wilt of tomato was significantly ($P = 0.05$) reduced by the chemical inducers, antagonistic bacteria, or their combination (Fig.1). For SB, highest inhibition was obtained by the combination of SB at 40 mg mL⁻¹ with either *P. macerans* MB02-992 or *P. polymyxa* MB02-1007 which reduced disease incidence by 72.20 and 74.02%, respectively, compared to the pathogen-infected plants (Fig. 1a). For As A, the highest inhibition was effected by the combination of As A at 8 mg mL⁻¹ with either *P. macerans* MB02-992 or *P. polymyxa* MB02-1007 which reduced disease incidence by 84.88% and 88.49% respectively, compared to the pathogen-infected plants (Fig. 1b). For INA, the highest inhibition was obtained by the combination of INA at 3 mg mL⁻¹ with either *P. macerans* MB02-992 or *P. polymyxa* MB02-1007 which reduced disease incidence by 89.12% and 92.24%, respectively, compared to the pathogen-infected plants (Fig. 1c). This is the first report on the use of SB, As A and INA, alone or in combination with antagonistic bacteria, in the control of tomato wilt and plant growth promotion. Algam *et al.*, 2010 reported that *P. polymyxa* MB02-1007 significantly increased the accumulation of pathogenesis-resistance proteins such as β -1,3-glucanase and chitinase in the presence of *R. solanacearum*. Therefore, the biocontrol effect of the two antagonistic bacteria may be attributed to both direct antagonism and induced resistance in tomato plants.

Results from this study indicated that the *in vitro* growth of *R. solanacearum* was significantly inhibited by the three chemical inducers, indicating that the chemical inducers may also be able to control bacterial wilt of tomato plants by direct antagonism. However, our findings are different from the results of Bigirimana and Hofte (2002), who reported that these chemical inducers have no role to play in the direct suppression of the pathogens which may be attributed to differences between pathogens.

In an earlier report, Algam *et al.* (2010) demonstrated the induction of systemic resistance by the exogenous application of various plant growth promoting rhizobacteria and chemical elicitors to plants against a range of bacterial pathogens. In particular, several studies have revealed that SB, As A and INA have been involved in the mechanism of biological resistance in plants (Malolepsza 2005; Barth *et al.*, 2006). It is probable that induction of systemic resistance is also involved here. The content of As A in plant tissues has been found to be associated with resistance to some diseases El-Gamal *et al.* (2007). Furthermore, application of SB under saline conditions has been recommended as a good hydroxyl scavenger which positively showed a slight increase in the activity of the enzyme superoxide dismutase, thus protecting the plant against oxidative damage (Gaballah and Gomaa, 2005). In addition, Malolepsza (2005) reported that the pretreatment of cucumber with INA before pathogen infections reduced the development of *Colletotricum lagenarium* infection.

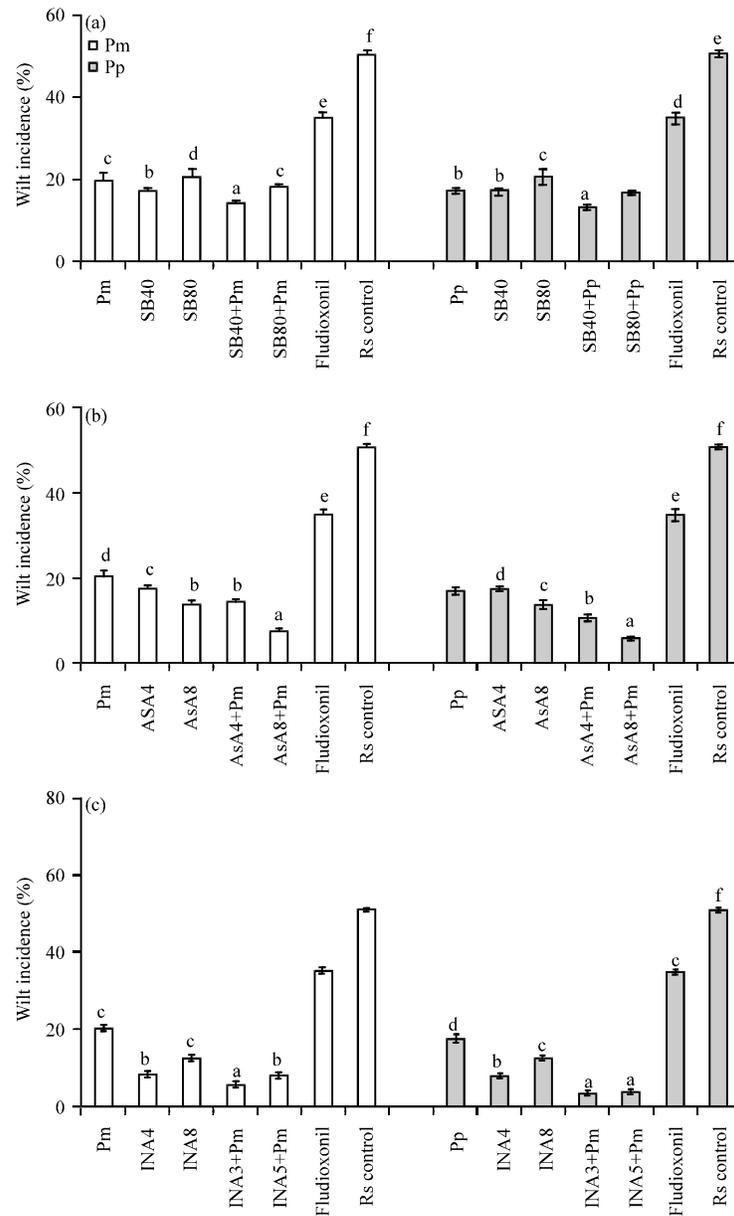


Fig. 1(a-c): Effect of antagonistic bacteria, chemical inducers or their combination on wilt incidence in tomato plants inoculated with *Ralstonia solanacearum* Rs-f.91 under greenhouse conditions. Disease incidence was scored at 14, 28 and 42 days after inoculation of *R. solanacearum*. Data from the repeated experiment were pooled and subjected to analysis of variance. Tomato plants uninoculated with *R. solanacearum* were free of symptoms and were not included in the statistical analysis. Means in a column followed by the same letter are not significantly different according to LSD test ($P = 0.05$). Pm = *Paenibacillus macerans*; Pp = *Paenibacillus polymyxa*; SB 40 and 80 = Sodium Benzoate at 40 and 80 mg mL⁻¹; As A 4 and 8 = Ascorbic Acid at 4 and 8 mg mL⁻¹; INA 3 and 5 = Isonicotinic Acid at 3 and 5 mg mL⁻¹; Rs = *Ralstonia solanacearum*

Population of *R. solanacearum* on tomato plants: The surviving cell numbers of *R. solanacearum* in tomato plants inoculated with the pathogen alone were 8.95 Log₁₀ CFU g⁻¹

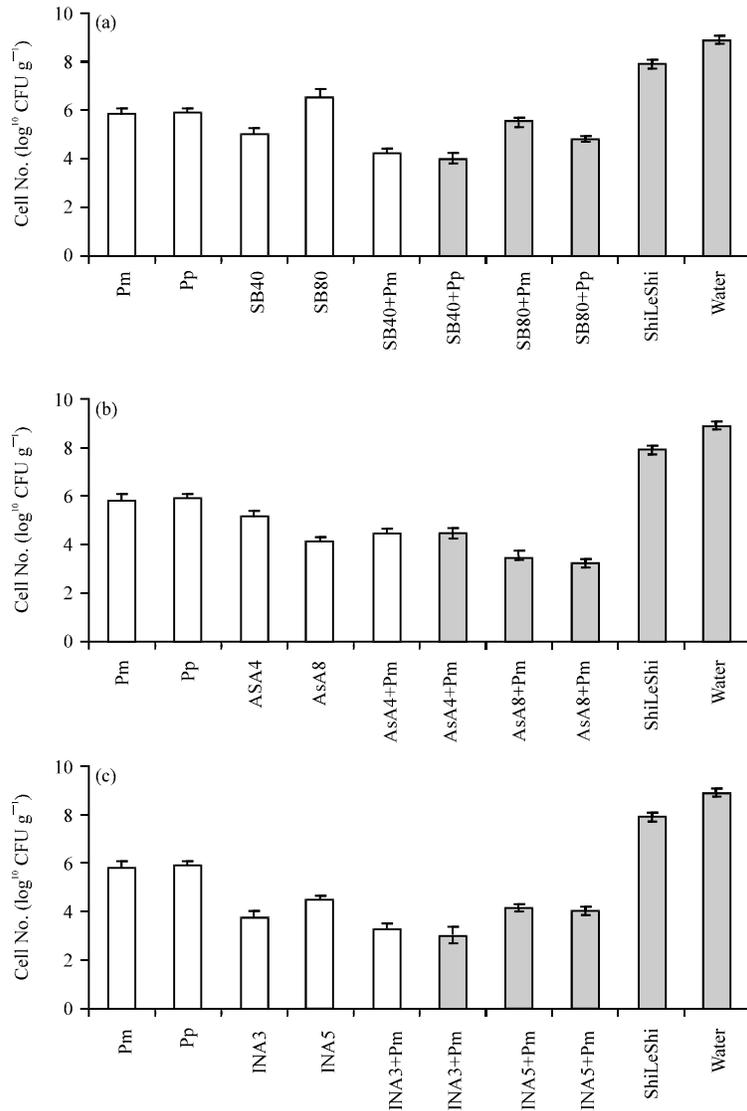


Fig. 2(a-c): Effect of antagonistic bacteria, chemical inducers or their combination on the population of *Ralstonia solanacearum* in stem tissues of tomato plants under greenhouse conditions. Data from the repeated experiment were pooled and subjected to analysis of variance. Error bars represent SE values and columns marked with the same letter are not significantly different from the control according to LSD test ($p = 0.05$). Pm = *Paenibacillus macerans*; Pp = *Paenibacillus polymyxa*; SB 40 and 80 = Sodium Benzoate at 40 and 80 mg mL⁻¹; As A 4 and 8 = Ascorbic Acid at 4 and 8 mg mL⁻¹; INA 3 and 5 = Isonicotinic Acid at 3 and 5 mg mL⁻¹; Rs = *Ralstonia solanacearum*

(Fig. 2). However, the cell numbers of *R. solanacearum* were significantly reduced after seeds were treated with either the chemical inducers or antagonistic bacteria (alone, or in combination) compared to the pathogen-infected plants (Fig. 2). For SB, the highest inhibition was obtained by the combination of SB at 40 mg mL⁻¹ with either *P. macerans* MB02-992 or *P. polymyxa*

MB02-1007 which reduced the surviving cell numbers by $4.15 \log_{10} \text{CFU g}^{-1}$ and $4.07 \log_{10} \text{CFU g}^{-1}$, respectively, compared to the pathogen-infected plants (Fig. 2a). For As A, best inhibition was obtained by the combination of As A at 8 mg mL^{-1} with either *P. macerans* MB02-992 or *P. polymyxa* MB02-1007 which reduced the surviving bacterial counts by $3.42 \log_{10} \text{CFU g}^{-1}$ and $3.32 \log_{10} \text{CFU g}^{-1}$, respectively, compared to the pathogen-infected plants (Fig. 2b). For INA, the highest inhibition was obtained by the combination of INA at 3 mg mL^{-1} with either *P. macerans* MB02-992 or *P. polymyxa* MB02-1007 which reduced the surviving cell numbers by $3.00 \log_{10} \text{CFU g}^{-1}$ and $2.88 \log_{10} \text{CFU g}^{-1}$, respectively, compared to the pathogen-infected plants (Fig. 2c). The reduction in population of *R. solanacearum* in tomato plants may explain the corresponding reduction of wilt incidence. This result is also consistent with the result of Lawton *et al.* (1996), who found that reduction in the severity of bacterial wilt of tomato plants was correlated with lower bacterial growth in treated plants as compared to the control plants. Similarly, Hassan and Buchenauer (2007) reported that biocontrol of fire blight by the combination of DL-b-amino-n-butyric acid with acibenzolar-s-methyl application should be attributed to the reduction of bacterial populations in apple seedlings.

In general, the combination of the chemical inducers and antagonistic bacteria significantly improved seed germination and seedling vigor, but the inhibition of tomato bacterial wilt and populations of *R. solanacearum* was more pronounced compared to either the chemical inducers or antagonistic bacteria alone. Interestingly, Rajkumar *et al.* (2008) reported that amendment with certain abiotic factors (inducers) appears to stimulate disease resistance by indirectly stimulating indigenous populations of microorganisms that are beneficial to plant growth and antagonistic to pathogens. Therefore, we suggest that the three chemical inducers may have had a beneficial effect on the growth of the two antagonistic bacteria.

Approaches to control bacterial wilt such as field sanitation, crop rotation and application of resistant varieties, have shown limited success. Bacterial wilt may not be managed with pesticides. Biological control, involving microbial agents or biochemicals, offers an eco-friendly and cost-effective alternative as an important component of an integrated disease management program (Algam *et al.*, 2010). Overall, this study indicated that chemical inducers, antagonistic bacteria, or both in combination have beneficial effects on tomato plant growth and health. In addition, the effect of the combination of the chemical inducers and antagonistic bacteria in tomato growth promotion and protection against bacterial wilt is in general significantly higher than that in presence of the chemical inducers or antagonistic bacteria alone.

CONCLUSION

This study has shown that chemical inducers, antagonistic bacteria, or their combination have beneficial effects on the growth and health of tomato plants.

The effect of the combination of chemical inducers and antagonistic bacteria on tomato growth promotion and protection against *Ralstonia solanacearum* wilt was significantly higher than that of the chemical inducers or antagonistic bacteria alone.

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