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Compatibility of Bio-control agents with bactericides for controlling bacterial wilt of tomato

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Abstract
A study was undertaken to evaluate the compatibility of bactericides at recommended dosage, half of the recommended dosage and one-fourth of the recommended dosage with *Trichoderma harzianum*, *Bacillus subtilis* and *Pseudomonas fluorescens* that are being used as biocontrol agents. On the basis of mycelial growth and spore germination in case of *T. harzianum* and on the basis of optical density in case of *B. subtilis* and *P. fluorescens* the tested bactericides streptomycin, ampicillin, chloramphenicol, copper hydroxide and chitosan were found compatible. Bleaching powder was found to be incompatible with fungal biocontrol agent whereas found to be compatible with bacterial biocontrol agents. Under glass house condition, all the tested bactericides except bleaching powder when used in combination with biocontrol agents were found effective in controlling bacterial wilt disease of tomato. Combination of bactericides with biocontrol agents was far more effective in controlling disease as compared to when used alone.

Keywords: Compatibility, Bactericides, *Trichoderma harzianum*, *Bacillus subtilis* and *Pseudomonas fluorescens*

Introduction

Tomato (*Solanum lycopersicum*) is the second most important and cultivated vegetable crop, next to potato around the world. It can easily be cultivated worldwide due to its easy adaptability to a wide range of climatic and soil conditions. India is the third largest producer of tomato in the world. Tomato is susceptible to more than 200 diseases. Among diseases, bacterial wilt is usually the most damaging causing about 60-70 percent yield loss. Bacterial wilt caused by the soil-borne plant pathogen *Ralstonia* solanacearum [9] is one of the most devastating bacterial plant diseases in the tropical and subtropical regions of the world. *Ralstonia solanacearum* gained its importance in the world due to its destructive nature, wide host range and geographical distribution. It affects a wide range of economically important crops such as tomato, potato, eggplant, chilli and non-solanaceous crops such as banana and groundnut in India. The bacterial wilt symptoms in tomato are characterized by initial wilting of upper leaves and within a few days followed by complete wilting of the plants. The vascular tissues of the infected stem have brown discoloration and if the stem is cut crosswise, white or yellowish bacterial ooze may be visible. Management of bacterial wilt in tomato and in other crops has been difficult. Integration of bio-agent with compatible chemicals may enhance the effectiveness of disease control and provide better disease management strategy to protect the seeds and seedlings from soil and seedborne inoculum [4]. Even reduced amount of chemicals can stress and weaken the pathogen and render its propagules more susceptible to subsequent attack by the antagonist [3]. Combining antagonists with synthetic and non-synthetic chemicals eliminates the chance of resistance development and reduces the chemical application. The biocontrol agent can be successfully combined with chemical methods of pest control to allow the population of biocontrol agent to overcome the pest population before it reaches to economic injury level. The synergistic phenomenon involved in approach of an integrated control, might be controlled more efficiently and for longer period by antagonist. Several systemic and contact fungicides were tested on basis of mycelial growth of *Trichoderma harzianum* and recorded that toxicity of the contact fungicides was lower than that of the systemic fungicides, among which copper oxychloride and copper hydroxide were highly compatible i.e. no inhibition was observed at lower concentration [5].

One chemical has been reported as compatible to a particular biocontrol agent in one study whereas other has reported otherwise. Further in recent past many molecules were found effective at relatively lower doses and their compatibility is yet to be worked out. Keeping in view the above facts, the present study was designed with an objective to study the compatibility between fungal and bacterial biocontrol agents and bactericides at different doses.

Materials and Methods

Source of bioagents

The biocontrol agents viz; *Trichoderma harzianum*, *Bacillus subtilis* and *Pseudomonas fluorescens* used in present investigation were obtained from Biocontrol Laboratory, Department of Plant Pathology, G.B Pant University of Agriculture and Technology, Pantnagar. The fungal biocontrol agent was cultured on Potato Dextrose Agar (PDA) medium and bacterial biocontrol agents on Nutrient Agar Medium (NA) and maintained at 27 ± 1ºC and 28± 1ºC respectively. Seven days old culture of *Trichoderma harzianum* and 2 days old culture of *Pseudomonas fluorescens* and *Bacillus subtilis* were used in all the studies.

Bactericides

Six bactericides viz.; streptomycin, ampicillin, chloramphenicol, bleaching powder, copper hydroxide and chitosan were tested at recommended, half of the recommended and one-fourth of the recommended dosage under *in vitro* and glass house conditions to check their compatibility with *T. harzianum*, *B.subtilis* and *P.fluorescens.*

In vitro evaluation

Estimation of compatibility of *Trichoderma harzianum* with bactericides

Effect of bactericides on inhibition of mycelial growth of *Trichoderma harzianum*

Different concentrations of bactericides i.e. recommended, half and one-fourth of the recommended dosage were evaluated to find out their effect on mycelial growth of *T. harzianum in vitro* using “Poison Food Technique” [6]. Five replications per treatment were maintained. The observation on radial growth of mycelium was taken by measuring the colony diameter (mm) vertically and horizontally at right angle and mean colony diameter was calculated. The percent inhibition in mycelial growth of *T. harzianum* over control was calculated.

Effect of bactericides on spore germination of *Trichoderma harzianum*

To find out the effect of tested bactericides on spore germination of *T. harzianum in vitro* “Cavity Slide Method” was used [1]. The spores of the test fungus were collected by washing 8 days old culture of *T. harzianum* on PDA plates with 10 ml distilled water and sterile suspension containing a spore concentration of 400 spores ml^{-1} was maintained by serial dilution and with the help of haemocytometer. Double strength concentration of bactericide was prepared in sterile distilled water. Equal volume (25 µl) of spore suspension and the chemical were added to the cavity slides to get desired concentrations. The spore suspension in sterile distilled water served as control. Each slide was kept in moist chamber prepared by putting moist blotter paper inside a petriplate. These plates were incubated in BOD incubator at 27±1ºC to facilitate conidial germination. The spore germination count in each treatment was recorded after 24 hrs of the experiment with the help of microscope. Five replications were kept for each treatment. The percent spore germination of *T. harzianum* strain over control was calculated.

Criteria of Compatibility

Four compatibility groups were made: Chemicals showing zero percent inhibition in mycelial growth or spore germination (Compatible), chemicals showing 1-50 percent inhibition in mycelial growth or spore germination (Moderately Compatible), chemicals showing 51-99 percent inhibition in mycelial growth or spore germination (Moderately Incompatible) and chemicals showing 100 percent inhibition in mycelial growth or spore germination (Incompatible) [8].

Estimation of compatibility of *Bacillus subtilis* and *Pseudomonas fluorescens* with bactericides

The compatibility of the bactericides with the bacterial antagonists viz; *P. fluorescens and B. subtilis* was tested using Turbidometric method [2]. One ml of the bacterial culture was transferred to a 250 ml sidearm flask containing 50 ml of Nutrient broth amended with bactericide. The control was maintained without inoculation of bacterial culture and bactericide technical standard in both the broth. The flasks were incubated at 28±2°C in a psycotherm shaker. The optical density values of the culture broth were determined in Spectrophoto meter at 610 nm at 24h.

Glasshouse Experiment

Six bactericides viz.; streptomycin, ampicillin, chloramphenicol, bleaching powder, copper hydroxide and chitosan at recommended, half and one-fourth of the recommended dosage were tested under glass house conditions to check their compatibility with *T. harzianum*, *B. subtilis* and *P. fluorescens* respectively. Twenty one days old tomato seedlings were transplanted in pots containing the mixture of vermicompost and soil (1:1). Bacterial cell suspension was prepared from 24hrs old culture of *Ralstonia solanacearum* and adjusted to 2× 10^8cells/ml (cfu) by adding sterilized distilled water and 0.7% (v/v) of Tween-40 (surfactant). One month old tomato plants were inoculated with pathogen by soil drenching method. The bactericides were added @ 100ml/pot, five days after pathogen inoculation. Second and third round of applications of treatments were given 10 and 20 days after the first application. Disease incidence was calculated and expressed in percentage scale, disease severity was calculated using 0-5 disease rating scale as described [12]. The Percent disease index (PDI) was worked out by using formula described by [11].

The data was analyzed statistically using Completely Randomized Design (CRD). The treatments were compared by the means of critical differences (CD) at 5% level of significance.

Results and Discussion

In vitro evaluation

Effect of bactericides on inhibition of mycelial growth of *Trichoderma harzianum*

The result revealed that the copper hydroxide at one-fourth of the recommended dosage had no effect on mycelial growth of *T. harzianum* as compared to control, thus found to be compatible. Streptomycin, ampicillin, chloramphenicol, chitosan and copper hydroxide even at half of the recommended dosage was found to be moderately compatible.

Bleaching powder even at one fourth of the recommended dosage significantly reduced the mycelial growth thus found to be moderately incompatible whereas bleaching powder at recommended dosage completely inhibited the mycelial growth of *Trichoderma harzianum* thus found to be incompatible with *T. harzianum* (Table 1).

Table 1: Effect of bactericides on radial growth of *Trichoderma harzianum*

Bactericides	**Concentration(ppm)**	**Colony Diameter (mm)**	**Inhibition of Mycelial growth (%)**
Streptomycin	64	65.80	26.88
Streptomycin	32	75.66	15.93
Streptomycin	16	80.16	10.93
Ampicillin	40	54.16	39.82
Ampicillin	20	64.33	28.52
Ampicillin	10	76.00	15.55
Chloramphenicol	13	53.50	40.55
Chloramphenicol	6.5	68.50	23.88
Chloramphenicol	3.2	73.83	17.96
Bleaching Powder	15000	0.00	100.00
Bleaching Powder	7500	11.00	87.77
Bleaching Powder	3750	38.16	57.60
Copper hydroxide	2000	51.16	43.15
Copper hydroxide	1000	80.66	10.37
Copper hydroxide	500	86.83	3.52
Chitosan	15000	63.33	29.63
Chitosan	7500	72.33	19.63
Chitosan	3750	75.33	16.30
Control	---	90.00	0.00
S.Em±	---	1.58	---
CD at 5%	---	4.81	---

The results obtained in the present investigation are in accordance with the previous findings indicating the safe nature of chitosan and copper hydroxide. Sharma *et al.* (2016) reported that chitosan at recommended dosage was found to be moderately compatible with *Trichoderma harzianum* Rifai strain PBAT-21 and Papavizas (1981) reported that *T.harzianum* was found to be compatible with copper hydroxide (Kocide 3000). No work has been done on the compatibility of these bactericides with fungal biocontrol agents under *in vitro* conditions except chitosan and copper hydroxide which were earlier tested. This is the first report about the compatibility of above bactericides with *T.harzianum* for controlling bacterial wilt disease in tomato.

Effect of bactericides on spore germination of *Trichoderma harzianum*

The effect of bactericides at recommended dosage, half of the recommended dosage and one-fourth of the recommended dosage was studied on spore germination of *T.harzianum* under *in vitro* conditions. All the tested bactericides were found to be moderately incompatible with *Trichoderma harzianum*. Review of literature revealed that no work has been done on the compatibility of these bactericides with *Trichoderma* spp. This is the first report about the effect of above bactericides on spore germination of *Trichoderma harzianum* (Table 2).

Table 2: Effect of bactericides on spore germination of *Trichoderma harzianum*

Bactericides	**Concentration(ppm)**	**Spore germination %**	**Spore germination Inhibition %**
Streptomycin	64	25.36	68.24
Streptomycin	32	26.13	67.28
Streptomycin	16	32.43	59.39
Ampicillin	40	19.40	75.70
Ampicillin	20	22.86	71.37
Ampicillin	10	24.76	68.99
Chloramphenicol	13	18.96	76.25
Chloramphenicol	6.5	21.46	73.12
Chloramphenicol	3.2	26.20	67.19
Bleaching Powder	15000	19.30	75.83
Bleaching Powder	7500	20.70	74.07
Bleaching Powder	3750	24.70	69.07
Copper hydroxide	2000	22.43	71.91
Copper hydroxide	1000	26.66	66.61
Copper hydroxide	500	27.63	65.40
Chitosan	15000	30.40	61.93
Chitosan	7500	31.03	61.14
Chitosan	3750	32.16	59.72
Control	---	79.86	0.00
S.Em±	---	2.03	---
CD at 5%	---	6.16	---

Estimation of compatibility of *Pseudomonas fluorescens* with bactericides

Data presented in Table 3 revealed that streptomycin, ampicillin at half and one-fourth of the recommended dosage, chloramphenicol, copper hydroxide and chitosan at one-fourth of the recommended dosage were found to be compatible whereas bleaching powder at all the tested concentration, chloramphenicol, copper hydroxide, chitosan at recommended and half of the recommended dosage and streptomycin and ampicillin at recommended dosage were found to be moderately compatible with *Pseudomonas fluorescens*.

The present results are in accordance with the findings of Papavizas *et al.* (1981) who reported that *Pseudomonas fluorescens* was found compatible with copper hydroxide (Kocide 3000) even at a high concentration of 300 ppm**.** No work has been done on the compatibility of these bactericides with bacterial biocontrol agents under *in vitro* conditions except copper hydroxide. This is the first report about the compatibility of above bactericides with bacterial biocontrol agents for controlling bacterial wilt disease in tomato.

Table 3: Effect of bactericides on growth of *Pseudomonas fluorescens*

Bactericides	Concentration(ppm)	O.D at 610 nm
Streptomycin	64	1.12
Streptomycin	32	1.27
Streptomycin	16	1.29
Ampicillin	40	1.11
Ampicillin	20	1.22
Ampicillin	10	1.24
Chloramphenicol	13	0.98
Chloramphenicol	6.5	1.11
Chloramphenicol	3.2	1.16
Bleaching Powder	15000	0.84
Bleaching Powder	7500	0.92
Bleaching Powder	3750	1.03
Copper hydroxide	2000	1.00
Copper hydroxide	1000	1.06
Copper hydroxide	500	1.38
Chitosan	15000	0.83
Chitosan	7500	1.03
Chitosan	3750	1.27
Control	---	1.36
S.Em±	---	0.06
CD at 5%	---	0.20

Estimation of compatibility of *Bacillus subtilis* with bactericides

The results revealed that no significant difference can be seen in bacterial growth, as indicated by the OD value of chitosan at all the tested concentration. Thus, chitosan at all the tested concentration, streptomycin and ampicillin at one-fourth of the recommended dosage were found compatible whereas bleaching powder, copper hydroxide, chloramphenicol at all the tested concentrations and streptomycin and ampicillin at recommended dosage and half of the recommended dosage were found to be moderately compatible with *Bacillus subtilis* (Table 4).

The present results are in accordance with the findings of Papavizas *et al.* (1981) who reported that *Bacillus subtilis* was found compatible with copper hydroxide (Kocide 3000) even at a high concentration of 300 ppm**.** No work has been done on the compatibility of these bactericides with bacterial biocontrol agents under *in vitro* conditions except copper hydroxide. This is the first report about the compatibility of above bactericides with bacterial biocontrol agents for controlling bacterial wilt disease in tomato.

Table 4: Effect of bactericides on growth of *Bacillus subtilis*

Bactericides	Concentration(ppm)	O.D at 610 nm
Streptomycin	64	1.13
Streptomycin	32	1.21
Streptomycin	16	1.27
Ampicillin	40	1.03
Ampicillin	20	1.21
Ampicillin	10	1.27
Chloramphenicol	13	1.10
Chloramphenicol	6.5	1.17
Chloramphenicol	3.2	1.21
Bleaching Powder	15000	0.73
Bleaching Powder	7500	0.82
Bleaching Powder	3750	0.90
Copper hydroxide	2000	0.81
Copper hydroxide	1000	0.86
Copper hydroxide	500	1.10
Chitosan	15000	1.35
Chitosan	7500	1.43
Chitosan	3750	1.46
Control		1.49
S.Em±	---	0.09
CD at 5%	---	0.27

Glass House Experiment

Estimation of compatibility of *Trichoderma harzianum* with bactericides

In glass house experiment, the tomato plants were treated with *T. harzianum* in combination with bactericides. Out of which *T. harzianum*+copper hydroxide was found to be most effective in reducing the disease severity followed by *T. harzianum*+chitosan, *T. harzianum* +ampicillin, *T. harzianum* +streptomycin and *T. harzianum* + chloramphenicol at all the tested concentration where as *T. harzianum* +bleaching powder was found to be least effective in controlling bacterial wilt disease as compared to treatment in which *Trichoderma harzianum* was used alone as well as control at 50 DAS. *T. harzianum* when used in combination with bactericides was far more effective in controlling the disease as compared to when used alone (Table 5).

Table 5: Effect of bactericides in combination with *T.harzianum* on bacterial wilt disease

Treatments	Concentration (ppm)	Percent Disease Index (PDI)		
		30 DAS	40 DAS	50 DAS
T.harzianum+Streptomycin	64	20.00	41.33	49.33
T.harzianum+Streptomycin	32	16.00	37.33	42.66
T.harzianum+Streptomycin	16	10.66	33.33	38.33
T.harzianum+Ampicillin	40	17.33	33.33	47.66
T.harzianum+Ampicillin	20	12.00	30.66	45.00
T.harzianum+Ampicillin	10	8.00	25.33	35.33
T.harzianum+Chloramphenicol	13	18.66	42.66	50.66
T.harzianum+Chloramphenicol	6.5	14.66	38.66	45.00

T.harzianum+Chloramphenicol	3.2	10.66	29.33	38.66
T.harzianum+Bleaching Powder	15000	34.66	61.33	73.66
T.harzianum+Bleaching Powder	7500	28.00	53.33	69.33
T.harzianum+Bleaching Powder	3750	25.33	50.66	62.00
T.harzianum+Copper hydroxide	2000	17.33	38.00	41.66
T.harzianum+Copper hydroxide	1000	13.33	37.33	41.33
T.harzianum+Copper hydroxide	500	10.66	24.00	29.33
T.harzianum+Chitosan	15000	20.00	42.66	47.66
T.harzianum+Chitosan	7500	14.66	34.66	40.33
T.harzianum+Chitosan	3750	9.33	26.66	30.00
T. harzianum	---	20.00	32.00	58.00
Control	---	51.00	67.00	86.33
S.Em±	---	1.69	3.33	2.62
CD at 5%	---	5.09	9.99	7.88

This is the first report about the compatibility of above bactericides with *T. harzianum* for controlling bacterial wilt disease in tomato.

Estimation of compatibility of *Pseudomonas fluorescens* with bactericides

Data presented in Table 6 revealed that *P. fluorescens* when used in combination with bactericides was found more effective in controlling disease at half and one-fourth of the recommended dosage as compared to *P. fluorescens* when used alone. *P. fluorescens* +copper hydroxide was found to be most effective in controlling bacterial wilt disease followed by *P. fluorescens*+streptomycin, *P. fluorescens* +ampicillin, *P. fluorescens* +chitosan, *P. fluorescens* +chloramphenicol where as *P. fluorescens* +bleaching powder was found to be least effective in controlling bacterial wilt disease. *P. fluorescens* when used in combination with bactericides at recommended doses was found ineffective in controlling the disease as compared to when *P. fluorescens* was used alone but was found to be effective when compared to control at 50 DAS.

Table 6: Effect of bactericides in combination with *P. fluorescens* on bacterial wilt disease

Treatments	**Concentration (ppm)**	**Percent Disease Index (PDI)**		
		30 DAS	**40 DAS**	**50 DAS**
P.fluorescens+Streptomycin	64	20.66	34.00	60.00
P.fluorescens+Streptomycin	32	14.66	29.33	44.00
P.fluorescens+Streptomycin	16	12.00	20.00	38.66
P.fluorescens+Ampicillin	40	17.00	39.33	64.00
P.fluorescens+Ampicillin	20	13.66	30.00	46.66
P.fluorescens+Ampicillin	10	9.33	18.66	37.33
P.fluorescens +Chloramphenicol	13	17.33	40.00	65.33
P.fluorescens +Chloramphenicol	6.5	12.00	30.66	54.66
P.fluorescens +Chloramphenicol	3.2	8.00	25.33	45.33
P.fluorescens+Bleaching Powder	15000	32.00	60.66	78.66
P.fluorescens+Bleaching Powder	7500	25.66	52.66	72.00
P.fluorescens+Bleaching Powder	3750	20.00	46.00	66.66
P.fluorescens+ Copper hydroxide	2000	16.66	37.33	57.33
P.fluorescens+ Copper hydroxide	1000	13.33	22.66	48.00
P.fluorescens+ Copper hydroxide	500	9.33	16.00	41.33
P.fluorescens + Chitosan	15000	16.00	40.66	60.00
P.fluorescens + Chitosan	7500	14.00	35.33	50.66
P.fluorescens + Chitosan	3750	10.66	25.33	45.33
P. fluorescens	---	24.00	36.00	52.00
Control	---	48.66	61.66	87.00
S.Em±	---	2.42	3.47	2.90
CD at 5%	---	7.25	10.40	8.71

This is the first report about the compatibility of above bactericides with *P.fluorescens* for controlling bacterial wilt disease in tomato.

Estimation of compatibility of *Bacillus subtilis* with bactericides

At one-fourth of the recommended dosage, combination of *B. subtilis* was found to be more effective in controlling bacterial wilt disease as compared to *B. subtilis* when used alone except *B. sublitis* when used in combination with bleaching powder. *B. subtilis*+copper hydroxide was found to be most effective followed by *B. subtilis*+chitosan, *B. subtilis*+streptomycin, *B. subtilis*+chloramphenicol and *B. subtilis*+ampicillin whereas *B. subtilis*+bleaching powder was found to be least effective as compared to *B. subtilis* when used alone for controlling bacterial wilt of tomato. *B. subtilis* when used in combination with bactericides at recommended doses was found ineffective in controlling the disease as compared to when *B. subtilis* was used alone but was found to be effective when compared to control at 50 DAS (Table 7).

Table 7: Effect of bactericides in combination with *B.subtilis* on bacterial wilt disease

Treatments	Concentration (ppm)	Percent Disease Index (PDI)		
		30 DAS	**40 DAS**	**50 DAS**
B.subtilis+Streptomycin	64	17.33	42.66	56.66
B.subtilis+Streptomycin	32	13.33	33.33	46.00
B.subtilis+Streptomycin	16	12.66	24.00	39.33
B.subtilis+Ampicillin	40	20.00	44.00	57.33
B.subtilis+Ampicillin	20	13.33	36.00	51.33
B.subtilis+Ampicillin	10	9.33	26.66	41.33
B.subtilis +Chloramphenicol	13	20.00	49.33	60.00
B.subtilis +Chloramphenicol	6.5	14.66	41.33	45.33
B.subtilis +Chloramphenicol	3.2	13.33	32.00	37.33
B.subtilis +Bleaching Powder	15000	29.33	65.33	80.33
B.subtilis +Bleaching Powder	7500	24.33	58.66	73.00
B.subtilis +Bleaching Powder	3750	20.66	52.00	66.66
B.subtilis +Copper hydroxide	2000	18.66	34.66	60.00
B.subtilis +Copper hydroxide	1000	16.00	26.66	42.66
B.subtilis +Copper hydroxide	500	13.33	18.66	37.33
B.subtilis+Chitosan	15000	16.00	40.00	57.33
B.subtilis+Chitosan	7500	14.66	32.00	46.66
B.subtilis+Chitosan	3750	12.00	26.66	36.00
B.subtilis	---	16.00	44.00	52.00
Control	---	46.66	64.66	85.66
S.Em±	---	2.62	3.12	2.54
CD at 5%	---	7.88	9.37	7.64

This is the first report about the compatibility of above bactericides with *B.subtilis* for controlling bacterial wilt disease in tomato.

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