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Studies on Compatibility of *Bacillus subtilis* (Ehrenberg) Cohn. with Chemical Fungicides

H. Basamma and Shripad Kulkarni*

Institute of Organic Farming, UAS Dharwad-580 005, Karnataka, India
**Corresponding author*

A B S T R A C T

The compatibility of bacterial biocontrol agent *Bacillus subtilis* was assessed with commonly used chemical fungicides viz., Carbendazim, Mancozeb, MetalaxylMZ, Wettable sulpher, Hexaconazole, Difenconazole, Tebuconazole and Kresoxim methyl. The compatibility was assessed at different concentrations and the concentration of 50, 100, 250, 500, 1000, 2000, 3000 and 5000 mg/lit for solid formulation fungicides and µl/lit for liquid formulation fungicides respectively. The compatibility tests revealed that among the solid formulation fungicides, the *B. subtilis* showed more tolerance to Carbendazim amd among the liquid formulation fungicides Hexaconazole and Kresoxim methyl showed maximum compatibility upto 3000µl/ l concentration. The fungicides *viz.*, Carbendazim, Difenconazole, Hexaconazole and Kresoxim methyl compatible with *B. subtilis* at concentrations which were recommended for plant disease management *i.e.*1000 mg Carbendazim/l, 500 µl Difenconazole/l and 1000 µl each of Kresoxim methyl and Hexaconazole/l seem to be safe tolerance limit for *B. subtilis*. Moreover, the pesticide tolerance ability broadened the use as these biopesticides in conjugation with pesticides can be applied under integrated disease management for the management of soil borne plant pathogens.

Keywords

Compatibility, *Bacillus subtilis*, Fungicides.

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Introduction

Plant pathogens are destructive and cause tremendous yield losses to all kinds of crops. Control of plant diseases by the use of antagonistic microorganisms can be an effective means (Cook and Baker, 1983). Interaction between biocontrol agents and plant pathogens has been studied extensively and application of biocontrol agents to protect some commercially important crops is promising (Vesseur *et al*., 1990). A large number of plant diseases have been successfully controlled through fungal and bacterial antagonists (Sahebani and Hadavi, 2008; Federico *et al*., 2007; Cook and Baker, 1983; Campbell, 1989; Vidhyasekaran *et al*., 1997). Supplementation with specific compounds may provide a competitive advantage for the establishment of the introduced biocontrol agents and improve the biocontrol. In several disease management strategies, the addition of fungicide at reduced rates in combination with biocontrol agents has significantly enhanced disease control, compared to treatments with biocontrol agent alone (Frances *et al*., 2002; Buck, 2004). Integrated use of biocontrol agent with reduced dose of fungicide was effective against many plant diseases compared with the individual components of disease management. The objectives of the present study is to test the growth of biocontrol agent *Bacillus subtilis* with commonly used pesticides at different concentrations under *in vitro* conditions for the control of plant pathogens.

Materials and Methods

Four solid formulation fungicides *viz.*, Wettable sulphur (Wet sulf 80WP), Carbendazim (Bavistin 50 WP), Mancozeb 75 WP (Indofil M 45), Metalaxyl MZ (Ridomyl MZ 72 WP) and four liquid formulation fungicides *viz.,* Hexaconazole (Contaf 5EC), Kresoxim-methyl (Ergon 44.3% SC), Difenconazole (Score 25% EC), Tebuconazole (Folicur 250EC) were tested against the *Bacillus subtilis* using poison food technique.

The different concentrations of the pesticides from 50, 100, 250, 500, 1000, 2000, 3000 and 5000 mg/l for solid formulation fungicides and µl/l for liquid formulation fungicides were prepared in nutrient agar. Desired concentration is poured in Petriplates and left over night to observe contamination if any. There after 0.1 ml of overnight culture of *B. subtilis* was spread over the solidified plates with spreader. These plates were incubated at 30±2 ^{0}C and *B. subtilis* colonies were identified and counted after 24h.

The observations on growth of *B. subtilis* on media containing different concentrations of various chemicals were recorded and maximum tolerance concentration (MTC) and maximum inhibition concentration (MIC) of fungicides for *B. subtilis* were calculated.

Where, MTC- Maximum or safe tolerance concentration ≤ 50 per cent reduction in cfu over control

MIC- Maximum inhibition concentration ≥ 90 per cent reduction in cfu over control

Results and Discussion

It is essential to test the compatibility of *Bacillus. subtilis* with the commonly used fungicides for their successful integration under IDM strategy of crop protection. Therefore, studies were undertaken on these aspects. The compatibility tests revealed that among the solid formulation fungicides, the *B. subtilis* showed more tolerance to Carbendazim as compared to other fungicides used in the study. It is compatible with *B. subtilis* up to 3000 mg/l concentration followed by Wettable sulphur and least in Mancozeb (Table 1, Plate 1and Fig. 1).

Among the liquid formulation fungicides tested Hexaconazole and Kresoxim methyl showed maximum compatibility upto 3000µl/ l concentration followed by tebuconazole and least in difenconazole (Table 2, Plate 2 and Fig. 2). The fungicides *viz.,* Carbendazim, Difenconazole, Hexaconazole and Kresoxim methyl compatible with *B. subtilis* at concentrations which were recommended for plant disease management *i.e.*1000 mg Carbendazim/l, 500 µl Difenconazole/l and 1000 µl each of Kresoxim methyl and Hexaconazole/l seem to be safe tolerance limit for *B. subtilis*. The results of this study showed combination of fungicides with *B. subtilis* can be used for seed treatment considering the reduction of bioagent population in combination and thus increasing the dosage of bioagent in seed treatment would be more helpful in suppression of plant diseases and thus the incompatibility constraint can be minimized. Similar reports were made by Mohiddin and Khan (2013) with respect to tolerance of fungal and bacterial agents to six pesticides commonly used in the control of soil borne plant pathogens.

Table.1 Compatibility of *Bacillus subtilis* with different solid formulation fungicides

Fungicide	Concentration (mg/l)																Mean
	Mean cfu count ($1x10^8$)/ml								Per cent reduction in mean cfu								
	50	100	250	500	1000	2000	3000	5000	50	100	250	500	1000	2000	3000	5000	
Carbendazim 50% WP	81.33	74.33	67.33	60.67	51.67	41.33	26.00	0.00	12.23 (20.46)*	19.78 (26.40)	27.34 (31.51)	34.53 (35.97)	44.24 (41.68)	55.39 (48.07)	71.28 (57.99)	100.00 (89.96)	45.59 (44.00)
Mancozeb 75% WP	57.00	51.67	46.00	38.00	25.00	14.33	0.00	0.00	38.48 (38.32)	44.25 (41.68)	50.35 (55.18)	58.99 (50.16)	73.03 (58.69)	84.52 (66.8)	100.00 (73.25)	100.00 (89.96)	68.70 (59.25)
Metalaxyl MZ 72% WP	65.67	58.67	53.33	46.67	37.33	22.33	13.33	0.00	29.13 (32.65)	36.69 (37.26)	42.45 (40.64	49.64 (44.77)	59.71 (50.58)	75.91 (60.58)	91.78 (67.68)	100.00 (89.96)	60.66 (53.01)
Wettable sulphur 50% WP	72.67	64.30	58.67	51.67	46.33	38.00	16.67	0.00	21.58 (27.67)	30.57 (33.57)	36.69 (37.26)	46.39 (42.91)	49.98 (44.98)	58.99 (50.16)	82.01 (64.88)	100.00 (89.96)	53.20 (97.82)
Control	92.67	**Mean**							**25.35 (29.77)**	**32.82 (34.72)**	**39.02 (41.12)**	**47.38 (43.46)**	**56.74 (48.98)**	**68.70 (56.40)**	**86.26 (65.95)**	**100 (89.96)**	

Comparing of means	S.Em±	C.D. at 1%
Fungicide (A)	0.56	1.76
Concentration (B)	0.46	1.44
Interaction (A x B)	0.61	1.90

* Arcsine transformed values

Table.2 Compatibility of *Bacillus subtilis* with different liquid formulation fungicides

Fungicide	Concentration (µl/l)																Mean
	Mean cfu count ($1x10^8$)/ml								Per cent reduction in mean cfu								
	50	**100**	**250**	**500**	**1000**	**2000**	**3000**	**5000**	**50**	**100**	**250**	**500**	**1000**	**2000**	**3000**	**5000**	
Difenconazole 25% EC	67.00	61.67	53.00	43.33	40.67	21.00	4.67	0.00	26.91 (31.24)*	32.73 (34.88)	42.18 (40.49)	48.37 (44.05)	55.64 (48.22)	77.09 (61.38)	94.91 (76.93)	100.00 (89.96)	59.73 (53.39)
Hexaconazole 5% EC	77.67	71.33	62.67	56.67	49.00	30.00	11.67	0.00	15.28 (23.00)	22.08 (28.09)	31.64 (34.21)	38.64 (38.15)	46.55 (43.00)	67.27 (55.08)	87.27 (69.07)	100.00 (89.96)	51.09 (45.57)
Kresoxim-methyl 44.3% SC	71.00	66.00	57.00	51.00	46.33	24.33	7.33	0.00	22.55 (28.34)	28.00 (31.94)	37.82 (37.94)	44.00 (41.54)	49.46 (44.67)	73.46 (58.96)	92.00 (73.54)	100.00 (89.96)	55.91 (50.86)
Tebuconazole 250% EC	65.00	57.33	52.00	43.67	34.33	12.67	0.00	0.00	29.09 (32.63)	37.46 (37.72)	43.27 (41.12)	52.37 (46.34)	62.15 (52.25)	86.18 (68.15)	100.00 (89.96)	100.00 (89.96)	62.94 (57.21)
Control	91.67							**Mean**	**23.45 (28.80)**	**30.06 (33.15)**	**38.72 (38.44)**	**45.84 (42.82)**	**53.45 (47.03)**	**76.00 (60.89)**	**93.80 (79.88)**	**100.00 (89.96)**	

Comparing of means	S.Em±	C.D. at 1%
Fungicide (A)	0.48	1.55
Concentration (B)	0.34	1.04
Interaction (A x B)	0.44	1.38

* Arcsine transformed values

Table.3 Maximum tolerance concentration (MTC) and Maximum inhibition concentration (MIC) of fungicides with *Bacillus subtilis*

Treatment	***Bacillus subtilis***	
	MTC	**MIC**
Solid formulation fungicides (mg/l concentration)		
Carbendazim 50% WP	1000	5000
Mancozeb 75% WP	250	3000
Metalaxyl MZ 72% WP	500	5000
Wettable sulphur 50% WP	1000	3000
Liquid formulation fungicides (µl /l concentration)		
Difenconazole 25% EC	500	3000
Hexaconazole 5% EC	1000	5000
Kresoxim-methyl 44.3% SC	1000	3000
Tebuconazole 250% EC	250	3000

Where MTC $\leq$ 50 per cent reduction in cfu
MIC $\geq$ 90 per cent reduction in cfu

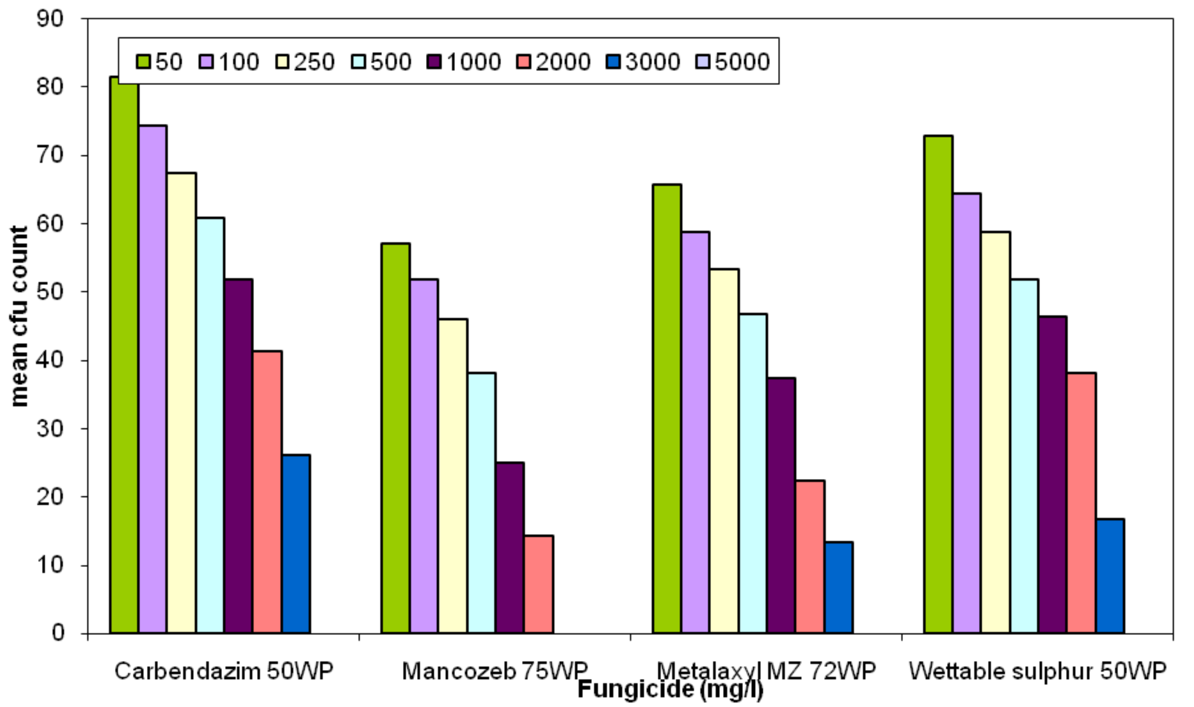


Fig. 1: Compatibility of *Bacillus subtilis* with different solid formulation fungicides

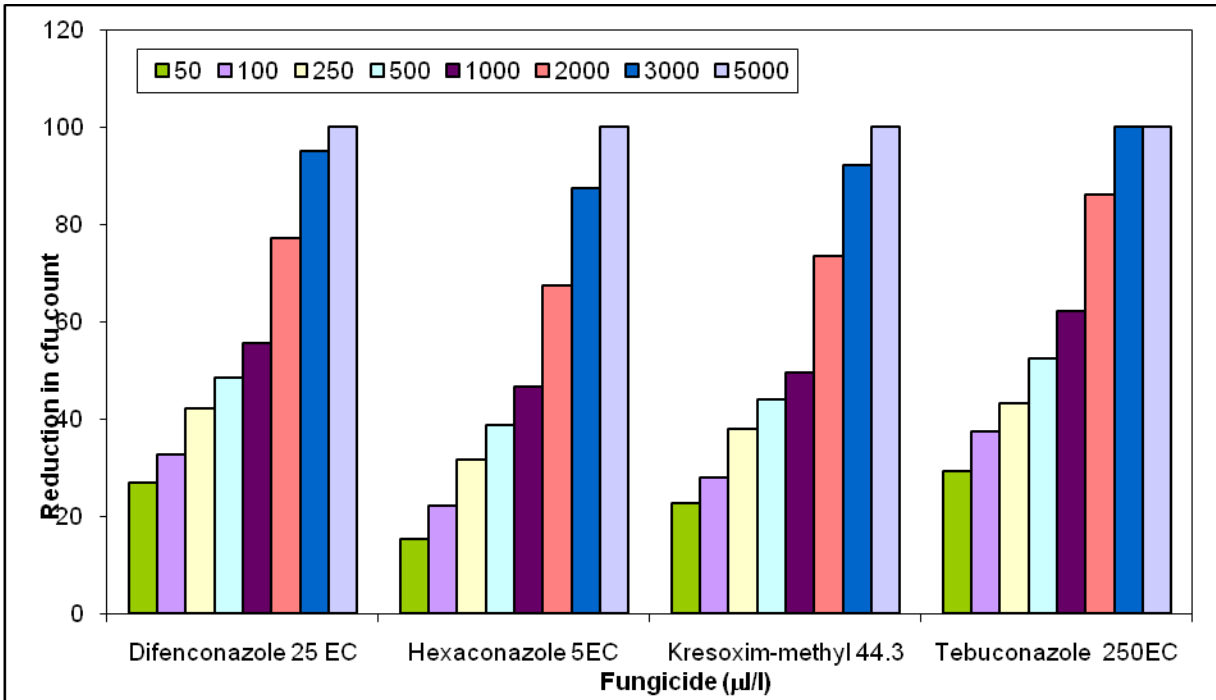
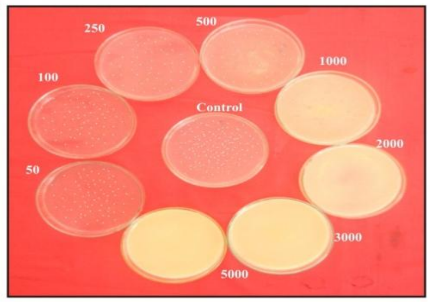


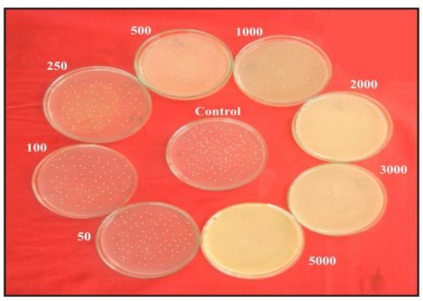
Fig. 2: Compatibility of *Bacillus subtilis* with different liquid formulation fungicides

Plate.1

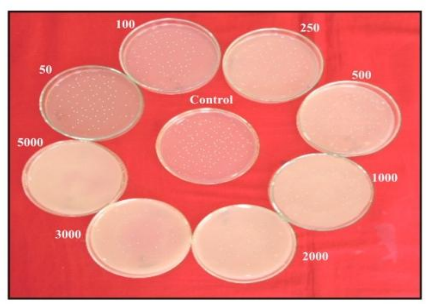
a. Carbendazim (mg/l)



b. Mancozeb (mg/l)



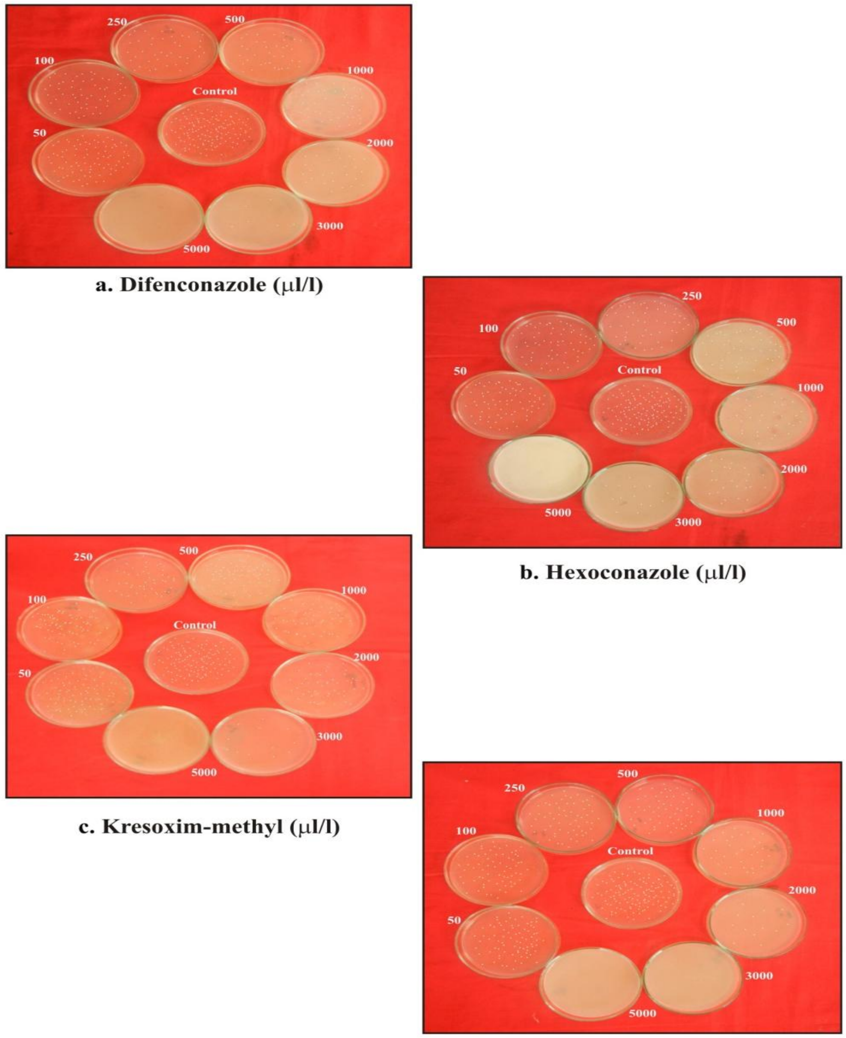
c. Metalaxyl MZ (mg/l)



d. Wettable sulphur (mg/l)

Plate 11: Compatibility of *B. subtilis* with solid formulation fungicides

Plate.2



a. Difenconazole (µl/l)

b. Hexoconazole (µl/l)

c. Kresoxim-methyl (µl/l)

d. Tebuconazole (µl/l)

Plate 12: Compatibility of *B. subtilis* with liquid formulation fungicides

They evaluated at different concentrations and reported that maximum tolerance concentration for *B. subtilis* were maximum in Carbendazim (5000mg/l) followed by Captan (3200 mg/l) whereas, Mancozeb was most inhibitory and supporting the findings of present study. Ahiladevi and Prakasam (2013) reported that *B. subtilis* was compatible with Azoxystrobin 25 EC at 5, 10, 50, 100 and 250 ppm concentrations. Vijaykrishna *et al.*, (2011) reported that *B. subtilis* strain mb1 600 was compatible with 1000 ppm of Hexaconazole, Propiconazole and Validamycin (Table 3).

In conclusion, Mancozeb, Metalaxyl MZ, Tebuconazole were found to be less compatible with *B. subtilis.* Whereas, Carbendazim, Hexaconazole and Difenconazole showing maximum tolerance limit upto their recommended concentration for disease management and showing high compatibility with *B. subtilis*.

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