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Compatibility *Bacillus subtilis* (BS 16) with fungicides used in chilli ecosystem for integrated disease management

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Abstract
Rhizosphere bacteria are one of the most potential biocontrol agents in plant disease management. Among them, *Bacillus subtilis* have advantages over other bacteria because of their ability to form endospore and broad spectrum antibiotic activities. The potential isolates of *B. subtilis* are identified on basis of bioefficacy and shelf. The population of *B. subtilis* (BS16) was significantly higher in talc powder (1.8×10^8cfu/g). The isolate *B. subtilis* (BS16) was studied for compatibility with commonly used fungicides of chilli ecosystem at four different concentrations, Among six fungicides, carbendazim 50 WP showed least zone of inhibition (15.03 mm) at 0.3 % and Tricyclazole 18 % + Mancozeb 62 % WP showed highest zone of inhibition (23.13 mm) at 0.3 %. It is concluded that carbendazim 50 WP is more compatible with *B. subtilis* compared to others fungicides. Hence *B. subtilis* (BS16) used as one of the component in integrated disease management.

Keywords: *Bacillus subtilis, Fungicides,* Compatibility and Bio agents

Introduction
Among the PGPR microbes, *Bacillus subtilis* is the one which plays a major role in plant growth promotion (Glick, 1995) [1] and biocontrol of pathogens. *B. subtilis* is a gram positive, rod shaped, endospore forming bacteria. which helps to withstand extreme temperatures as well as dry environments. This endospore helps the organism to persist in the environment until conditions became favourable. For successful integration of bio control agents in Integrated Disease Management systems (IDM), it is pre-requisite for them to be compatible with chemicals *viz.*, fungicides antibiotics, insecticides and plant extracts are currently employed for the management of plant diseases. Hence screening of efficient biocontrol agents that are compatible with the chemical counterparts of IDM systems greatly helps in this direction.

The bioagents alone will not be lasting solution for management of diseases of any crops. In fact chilli is infected by several pathogens of fungi, bacteria, nematodes etc. In other words in an ecosystem the bioagent can well be integrated with IPM inputs, hence its compatibility with other bioagent is called for. Laha and Venkataraman (2001) [2] have noted the compatibility of *P. fluorescens* with carbendazium while studying sheath blight in rice. Rhizobacteria showing antagonism against *Aphanomyces cochloides* were tested for compatibility with thiram and hymexazol. Integration of *P. fluorescens* with thiram in pellet was found to be feasible. The effect of carbendazium and thiophanate methyl on growth of *T. viride* (Tv 33, Tv 48,and Tv 52), *T. harizianum* (Th 5 and Th 62) and *P. fluorescens* (ARR1G and VPY10 *in vitro*. Carbendazium and thiophanate methyl at 100 ppm enhanced the growth of *P. fluorescens* isolates and even at 500 ppm concentration antagonistic bacteria multiplied. Combined application of *P. fluorescens* + thiophanate methyl resulted in highest plant stand (Malathi *et al*., 2002) [3]. De *et al.* (2003) [4] conducted the pot and field experiments to evaluate the biocontrol potential of *T. harizianum*, *Gliocladium virens* and *P. fluorescens* against *F. oxysporum* f. sp. *lentis* infecting lentils and their compatibility with fungicides. In pots, pre sowing seed treatment (ST) with *P. fluorescens*+ carboxin resulted in 62.3 per cent wilt control. ST with carbendazium + thiram and *G. virens* + *P. fluorescens* + carboxin were effective in the field controlling 48.8 and 44.2 per cent respectively. Khan and Gangopadhyay (2008) [5] reported that compatibility of *P. fluorescens* with fungicides *in vitro*. carboxin, chlorothalonil and carbendazium were least toxic to *P. fluorescens* strain PFBC-25, while

captan was most inhibitory. The compatibility studies of *P. fluorescens* (Pf1) with azoxystrobin at concentrations *viz.,* 100, 150, 200, 250 and 300 ppm revealed that it was compatible with all the concentrations of azoxystrobin tested. The field experiment revealed that foliar application of combination of Pf1 (2.5 kg ha^{-1}) and azoxystrobin (250 ml ha^{-1}) reduced downy mildew and powdery mildew disease severities more than azoxystrobin (250 and 500 ml ha^{-1}) alone. Application of Pf1+azoxystrobin treatment recorded only 2.22 and 1.00 per cent disease index (PDI) of downy mildew and 1.85 and 0.50 PDI of powdery mildew during first and second seasons, respectively. The treatment also recorded a maximum fruit yield of 14.30 and 15.65 tonnes ha^{-1} for the first and second season respectively. Application of Pf1 along with azoxystrobin significantly increased the survival of Pf1 in the phylloplane of cucumber crop. In addition, there was increase in PO,PPO, PAL, β 1, 3 glucanase, chitinase and phenolics in plants treated with Pf1+azoxystrobin (Anand and Bhaskaran, 2009) [6].

Ongena *et al*. (2013) [7] evaluated the compatibility of azoxystrobin 25 SC with biocontrol agents by poisoned food technique under *in vitro* conditions. The results showed that *P. fluorescens* and *B. subtilis* were compatible with azoxystrobin 25 SC at 5, 10, 50, 100 and 250 ppm concentrations. Rajalaxmi *et al*. (2013) [8] studied the compatibility of fungicides with *P. fluorescens* and *P. putida.* Both the bioagents were compatible with carbendazium, hexaconozole and propiconazole at both 0.1 and 0.2 per cent concentrations. Both were incompatible with mancozeb and captan. An experiment was conducted to study the compatibility of copper hydroxide (Kocide 3000) with biocontrol agents under *in vitro* conditions. Bioagents *viz.*, *P. fluorescens* and *B. subtilis* were compatible with copper hydroxide (Kocide 3000) even at a high concentration of 300 ppm (Valarmathi *et al*., 2013) [9].

Once the antagonistic potential isolates of *B. subtilis* are identified, further the eco-system such as chilli is identified with multiple problems of diseases and insect pests. The PGPR alone will not be able to cater the management of diseases and insect pests.

Hence the proposed *B. subtilis* has to be integrated with other IPM inputs such as fungicides therefore its compatibility with IPM inputs was undertaken.

Materials and Methods

Isolates collection

Bacillus subtilis (BS16) was isolated from the rhizosphere soil by serial dilution and plate count technique and potential isolate was selected on basis of bio efficacy and their shelf life study (Pankaj Kumar *et al*., 2012) [10].

Fungicides

Carbendazim 50 WP (Kevisten), Tricyclazole 18 % + Mancozeb 62 % WP(Merger), Trifloxystrobin 25 % + Tebuconazole 50 % EC(Nativo), Zineb 68 % + Hexaconazole 4 % WP(Avatar), Carbendazim 12 % + Mancozeb 63 % WP(SAAF) and Hexaconozole 5 % EC(Contaf)

Media

Nutrient agar medium

Peptone: 10 g
NaCl: 5 g
Beef extract: 1.5 g
Yeast extracts: 1.5 g
Agar: 10 g
Distilled water: 1000 ml
Ph was adjusted to: 7.2

Technique

The modified paper disc technique is also called as "zone of inhibition technique" (Mohiddin *et al*., 2013) [11] was made use. This zone of inhibition was followed to evaluate the compatibility of the fungicides with potential isolate of *B. Subtilis* (BS-16). 20 ml of molten NA medium initially mixed with *B. subtilis* was poured in to 90 mm diameter Petri dishes after solidification, 5 mm discs of paper dipped in chemical was placed at the centre of the plate, control was maintained by distilled water dipped paper disc. The experiment was done using CRD (Completely Randomized Design) design. Each set of experiment was replicated thrice and plates were incubated at 28 ±1 °C for six days. Observations were taken on parameters such as zone of inhibition of colonies and colony diameter (Table 1).

Results and Discussion

The potential isolate of *B. subtilis* (BS-16) was studied for compatibility with six fungicides, Kevisten (carbendazim 50 WP), contaf (hexaconozole 5 % EC) native (trifloxystrobin 25 % + tebuconazole 50 % EC), avatar (zineb 68 % + hexaconazole 4 % WP), merger (tricyclazole 18 % + mancozeb 62 % WP) and SAAF (carbendazim12% + mancozeb 63 % WP) at four concentrations, 0.05, 0.1, 0.2 and 0.3 per cent. Among six fungicides carbendazim 50 WP showed least zone of inhibition (15.03 mm) at (0.3 %) (Table 1). Based on these results conclude that carbendazim 50 WP more compatible with *B. subtilis* compared to others followed by Carbendazim 12 % + Mancozeb 63 % WP at all four concentrations and (tricyclazole 18 % + mancozeb 62 % WP) showed highest zone of inhibition (23.13 mm at 0.3 %) and least compatible compared other fungicide (Plate 1) (Fig. 1).

The effect of carbendazium and thiophanate methyl on growth of *T. viride* (Tv 33, Tv 48 and Tv 52), *T. harizianum* (Th 5 and Th 62) and *P. fluorescens* was studied *in vitro*. Carbendazium and thiophanate methyl at 100 ppm enhanced the growth of *P. fluorescens* isolates and even at 500 ppm concentration antagonistic bacteria multiplied. Combined application of *P. fluorescens* + thiophanate methyl resulted in highest plant stand (Malathi *et al*., 2002) [12]. Ongena *et al*. (2013) [13] evaluated the compatibility of azoxystrobin 25 SC with biocontrol agents by poisoned food technique under *in vitro* conditions. The results showed that *P. fluorescens* and *B. subtilis* were compatible with azoxystrobin 25 SC at 5, 10, 50, 100 and 250 ppm concentrations. Valarmathi *et al*. (2013) [14] reported the compatibility of copper hydroxide (Kocide 3000) with biocontrol agents under *in vitro* conditions. Bioagents *viz.*, *P. fluorescens* and *B. subtilis* were compatible with copper hydroxide (Kocide 3000) even at a high concentration of 300 ppm.

Table 1: Compatibility of *B. subtilis* (BS16) with commonly used fungicides of Chilli Ecosystem

Treatment	Fungicides	Zone of inhibition (mm)			
		Concentrations			
		0.05 %	0.1 %	0.2 %	0.3 %
T1	Carbendazim 50 % WP	10.10	11.13	12.93	15.03
T2	Tricyclazole 18 % + Mancozeb 62 % WP	15.06	17.10	20.96	23.13
T3	Trifloxystrobin 25 % + Tebuconazole 50 % EC	15.01	17.40	19.06	21.03
T4	Zineb 68 % + Hexaconazole 4 % WP	14.02	17.06	19.03	21.03
T5	Carbendazim 12 % + Mancozeb 63 % WP	12.10	15.03	16.93	18.06
T6	Hexaconozole 5 % EC	15.06	17.01	18.96	19.93
T7	Control	00.00	00.00	00.00	00.00
	S.Em ±	0.34			
	C.D at 1 %	1.47			

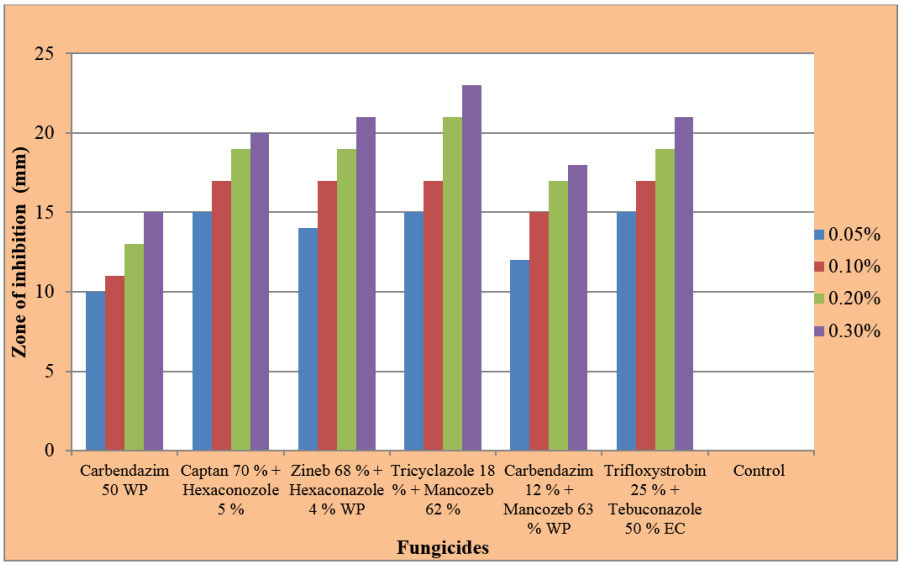
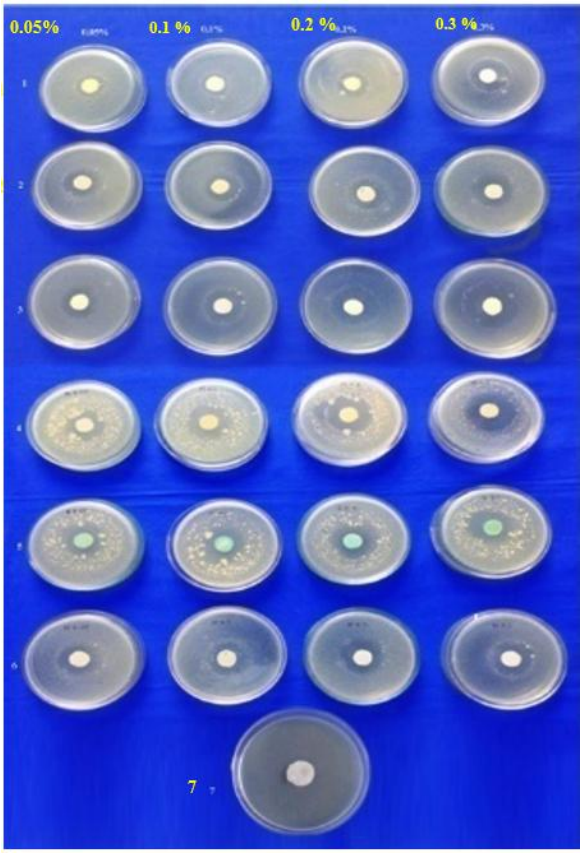


Fig 1: Compatibility of *B. subtilis* isolate (BS16) against commonly used fungicides

Compatibility of *B. subtilis* (BS 16) with commonly used fungicides of chilli Ecosystem



1. Carbendazim 50 % WP, 2. Hexaconozole 5 % EC, 3. Zineb 68 % + Hexaconazole 4 % WP, 4. Tricyclazole 18 % + Mancozeb 62 % WP, 5. Carbendazim 12 % + Mancozeb 63 % WP, 6. Trifloxystrobin 25 % + Tebuconazole 50 % EC, 7. Control

Conclusion

The population of *B. subtilis* (BS16) was significantly higher in talc powder and highly efficient strain showed the compatibility with Carbendazim 50 WP followed by Carbendazim 12 % + Mancozeb 63 % WP at 0.01%. Hence *B. subtilis* (BS16) has to be integrated with other IPM inputs in chilli ecosystem.

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