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Compatibility studies of *Bacillus subtilis* (Ehrenberg) Cohn. with commonly used fungicides

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Abstract

Bio-control agents could be used as an eco-friendly approach to effectively control the disease and may be advised to the farmers for profitable organic farming. Evaluation of different fungicides were tested with *Bacillus subtilis* under *in vitro* by modified paper disc technique or "zone of inhibition technique". The different fungicides *viz.*, carbendazim 50 WP, carboxin 75 WP, hexaconazole 5% EC, azoxystrobin 23 SC, ridomyl 4% + mancozeb 64% WP, trifloxystrobin 25% + tebuconazole 50% WG were evaluated at 500 and 1000 ppm. Among these fungicides evaluvated, carbendazim 50 WP and azoxystrobin 23 SC showed least zone of inhibition 09.16 mm and 9.33 mm at 500 ppm respectively. Carboxin 75 WP and ridomyl 4% + mancozeb 64% WP showed highest zone of inhibition 26.33 mm and 21.33 mm at 1000 ppm respectively. The results from the biological compatibility revealed that fungicides carbendazim 50 WP and azoxystrobin 23 SC were found to be more compatible with *Bacillus subtilis* (BS 6), when compared to other fungicides.

Keywords: Bacillus subtilis, fungicides, bio agents, compatibility

Introduction

In the present day agriculture, Integrated Pest Management (IPM) and Integrated Nutrient Management (INM) concepts are gaining importance with an ultimate aim of total crop care through the use of various inputs. Integrated Plant Disease Management (IPDM) is an approach that attempts to make complementary use of cultural, chemical, biological and host resistance methods of disease management for obtaining best possible results. One of the important components of IPDM is the use of antagonistic microorganisms, among which Bacillus subtilis, a bacterial biocontrol agent play an important role in plant diseases management and hence carring popularity. Biological control of plant pathogens by B. subtilis is a vital area of plant pathological research all over the world in present days as most of the plant diseases are not amenable for management through chemicals. The need for designing cultural and biological control is obvious and a distinct possibility in the framework of IPDM. Among the PGPR microbes, Bacillus subtilis (Cohn, 1872)^[2], is an adept rhizobacterium and has gained global attention as a biopesticide (Edgecomb and Manker, 2006)^[4] for the control of several plant diseases. It is known to inhibit a number of soil borne pathogens (Asaka and Shoda, 1996; Edgecomb and Manker, 2006)^[1, 4]. The potentiality of this biocontrol bacterium has been reported to be effective against soil borne pathogens (Asaka and Shoda, 1996; Edgecomb and Manker, 2006)^[1, 4]. The activity of the biocontrol agents is highly influenced by several factors. In agriculture. Application of agrochemicals is common worldwide to protect the crops against such kind of plant pathogens. In the present day of plant protection scenario, development of resistance to chemical pesticides is the major hurdle for disease management (Gerhardson, 2002)^[5], the chemicals also leads to various toxic effects to environment and side effects to human health (Martinez-Absalon et al., 2012)^[6].

Devi and Prakasam (2013)^[3] tested the compatibility nature of azoxystrobin 25 SC with *Pseudomonas fluorescens* and *Bacillus subtilis* on anthracnose of chilli. The results showed that *P. fluorescens* and *B. subtilis* were compatible with azoxystrobin 25 SC at 5, 10, 50, 100 and 250 ppm concentrations. Rajkumar *et al.* (2018)^[10].

conducted an experiment on compatibility of *Bacillus subtilis* (BS 16) with fungicides used in chilli ecosystem for integrated disease management. 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 was more compatible with *B. subtilis* compared to other fungicides under *in vitro*.

Search for effective biocontrol agents for the management of plant diseases has been intensified in recent years to reduce the dependence on ecologically hazardous chemicals. Biocontrol agents must be effective and compatible with latest crop production practices so that they can be integrated into the protection system. Compatibility of beneficial organisms with modern inputs in plant protection like fungicides, insecticides and weedicides is a pre-requisite for developing integrated disease management strategies. Hence, in the present study, the compatibility of *B. subtilis* with 6 fungicides was studied by *in vitro* method.

Materials and Methods Materials required

Isolates of *Bacillus subtilis*

Bacillus subtilis (BS6) was isolated from the rhizosphere soil by serial dilution and plate count technique and potential isolate was selected on basis of bio efficacy (Pankaj Kumar *et al.*, 2012)^[9].

Fungicides

Carbendazim 50 WP (Bavistin), Carboxin 75 WP (Vitavax), Hexaconazole 5% EC (Contaf), Azoxystrobin 23 SC (Amister), Ridomyl 4% + Mancozeb 64% WP (Ridomil gold), Trifloxystrobin 25% + Tebuconazole 50% WG (Nativo) were evaluated at 500 and 1000 ppm.

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

A study on compatibility of *Bacillus subtilis* with commonly used fungicides was conducted under laboratory conditions using modified paper disc technique also called as "zone of inhibition technique" (Mohiddin and khan, 2013) ^[7]. Each fungicide was evaluated at two concentrations *viz.*, 500 ppm and 1000 ppm. Twenty ml of molten nutrient agar medium initially mixed with *Bacillus subtilis* was poured in to

sterilized petri dishes. After solidification, 5 mm discs of paper dipped separately in the above mentioned fungicides and concentrations was placed at the centre of the plate. Control was maintained by placing the paper disc dipped in sterile distilled water. The experiment was done using CRD (Completely Randomized Design) design. Each treatment was replicated thrice and plates were incubated at 28 ± 2 °C for 3 days. Observations were drawn for the formation of zone of inhibition.

Results and Discussion

The results pertaining to the compatibility of *B. subtilis* with six fungicides are presented in Table 1. Among the six fungicides evaluated, none of fungicides found well compatible with the biocontrol agent as all the treated fungicides exhibited the property of inhibiting the growth of bicontrol agent, however, carbendazim 50 WP showed least zone of inhibition by 9.16 mm at 500 ppm concentration followed by azoxystrobin 23 SC at 500 ppm (9.33 mm). There was no significant difference in the inhibition of 25.33 mm was recorded in carboxin 75 WP treated plates at 500 ppm and 26.33 at 1000 ppm concentration, which significantly inhibited the growth of biocontrol agent (Plate 1 and Fig 1).

The present study results in respect of compatibility were in confirmly with the findings of Ongena (2013)^[8]. The author opined that the fungicide azoxystrobin 25 SC was compatible with *P. fluorescens* and *B. subtilis* at lower concentrations of 5, 10, 50, 100 and 250 ppm. Azoxystrobin exhibited the least inhibition of *B. subtilis* in the present investigation.

Mohiddin and Khan (2013)^[7] assessed the tolerance of fungal and bacterial biocontrol agents with commonly used six fungicides and observed the maximum tolerance of *B. subtilis* to carbendazim (5000mg/l) followed by Captan (3200 mg/l), whereas the most commonly used fungicide mancozeb was found most inhibitory to the biocontrol agent.

Table 1: Compatibility of B. subtilis with commonly used fungicides

	Zone of inhibition (mm)	
Chemicals	Concentration	
	500 ppm	1000 ppm
Carbendazim 50 WP	09.16	09.50
Carboxin 75 WP	25.33	26.33
Hexaconazole 5% EC	10.00	13.33
Azoxystrobin 23 SC	09.33	09.66
Ridomyl 4% + Mancozeb 64% WP	18.33	21.33
Trifloxystrobin 25% + Tebuconazole 50% WG	11.33	12.66
Control	00.00	00.00
Comparing of means	S.Em±	C.D at 1%
Fungicides (A)	0.84	3.32
Concentration (B)	0.48	1.92
Interaction (A x B)	1.19	4.70

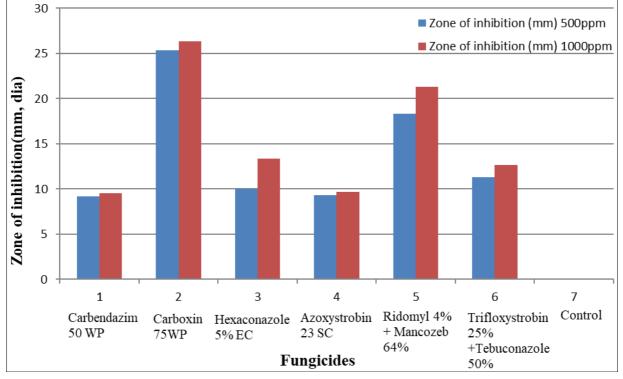
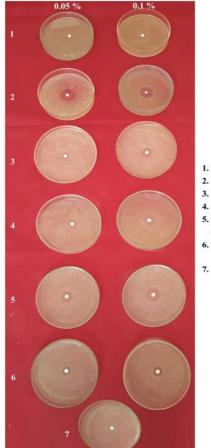


Fig 1: Compatibility of B. subtilis with commonly used fungicides



- 1. Carbendazim 50 WP,
- 2. Carboxin 75WP,
- 3. Hexaconazole 5 % EC,
- Azoxystrobin 23 SC,
 Ridomyl 4 % + Mancozeb
- 64 % WP, 6. Trifloxystrobin 25 % +
- Tebuconazole 50 % WG, 7. Control

Plate 1: Compatibility of B. subtilis with commonly used fungicides

Conclusion

Carboxin 75 WP and Ridomyl 4% + Mancozeb 64% WP were less compatible with *B. subtilis*. Whereas, Carbendazim 50 WP and Azoxystrobin 23 SC showing maximum tolerance limit upto their recommended concentration were having little bit compatibility with *B. subtilis*.

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