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

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DOI: [https://doi.org/10.22271/chemi.2020.v8.i4o.9833](https://doi.org/10.22271/chemi.2020.v8.i4o.9833)

**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 evaluated, 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 carrying 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) [3], 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 | 5 g |
| NaCl         | 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 biocontrol 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 concentration between these two fungicides. Highest 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 1and Fig 1). The present study results in respect of compatibility were in conformity 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 |
|-----------------|-----------------|-----------------|------------------|
| **Chemicals**   | **Zone of inhibition (mm)** |
|                 | **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.E.M±** | **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

| Fungicides          | Zone of inhibition (mm) 500ppm | Zone of inhibition (mm) 1000ppm |
|---------------------|---------------------------------|----------------------------------|
| Carbendazin 50 WP   | 30                              | 25                               |
| Carboxin 75 WP      | 25                              | 20                               |
| Hexaconazole 5% EC  | 20                              | 15                               |
| Azoxystrobin 23 SC | 15                              | 10                               |
| Ridomyl 4% + Mancozeb 64% |                  |                                  |
| Trifloxystrobin 25% + Tebuconazole 50% |            |                                  |
| Control             | 0                               | 0                                |

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 up to their recommended concentration were having little bit compatibility with B. subtilis.

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