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# COMPATIBILITY OF *Bacillus subtilis* WITH DIFFERENT NOVEL FUNGICIDES USED AGAINST PLANT PATHOGENS

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## ABSTRACT

***Bacillus* species are known for their broad-spectrum antimicrobial activity and have gained significant interest as bio-control agents in agriculture. Now a day's use of new chemicals for crop management is also governing the growth and performance of bioagents. Having a tolerant strain with all desirable characters, its multiplication and application is a limiting factor for use of biocontrol agents. An experiment was conducted during 2022-2023 at laboratory of Plant Pathology, Barrister Thakur Chhedilal College of Agriculture and Research Station, Bilaspur, Indira Gandhi Krishi Vishwavidyalaya, Raipur (Chhattisgarh) to study the compatibility of *Bacillus subtilis* with different novel fungicides used against plant pathogens under *in vitro* condition. The compatibility of fungicides at five different concentrations with *B. subtilis* was studied and it was found that Carbendazim 50% WP, Azoxystrobin 25% SC, Propiconazole 10.7% + Tricyclazole 34.2% SE, Azoxystrobin 18.2% + Difenoconazole 11.4% SC, Tebuconazole 50% + Trifluoxystrobin 25% WG, Trifloxystrobin 25% WG, Fosetyl Al 80% WP and Azoxystrobin 18.2%+Cyproconazole 7.3% W/Vwere found highly compatible with *B. subtilis* at all concentrations (100, 500, 1000, 1500, 2000 ppm) and recorded higher number of colony forming units (CFU's) and can be used under the field condition for the control of soil borne and foliar diseases. Whereas, Azoxystrobin 7.1% + Propiconazole 11.9% SE and Fluxapyroxad 6.25 %+ Epoxiconazole 6.25% SC were found inhibitory at higher concentrations (1000, 1500 and 2000 ppm). Fungicides i.e. Hexaconazole 50% SC, Mancozeb 75% WP, Carbendazim 12% + Mancozeb 63%WP, Chlorothaonil 75%WP and Picoxystrobin 7.05% + Propiconazole 11.9% SE were completely inhibitory even at 100 ppm concentration. Moreover, Azoxystrobin 25% W/W followed by Fosetyl Al 80% WP and Carbendazim 75% WP were found to be highly compatible with *Bacillus subtilis* and had higher colony forming units (CFU's) which were at par with control at 100 and 500 ppm concentration.**

**(Key words: Fungicides, *Bacillus subtilis*, pathogens and bioagent)**

## INTRODUCTION

*Bacillus subtilis* is an antagonistic bacterial biological agent which controls many air borne, seed borne and soil borne diseases (Akhond *et al*., 2016). Extensive research indicates that the effects of *Bacillus subtilis* on plant disease suppression through various mechanisms including antibiosis, hyphallysis of pathogens, competition for nutrients and space. Moreover, it has been found that *Bacillus subtilis* can induce systemic resistance (ISR) in plants enhancing their ability to defend against diseases (Cao *et al.*, 2011; Yu *et al.*, 2011; Li *et al.,* 2013). The genus *Bacillus* is classified in the order Eubacteriales and family Bacillaceae. *Bacillus subtilis* was initially discovered by Ehrenberg in 1835 and was initially referred to as *Vibrio subtilis.* However, it was renamed by Cohin 1872. *Bacillus subtilis* is a gram-positive, rod-shaped bacterium with peritrichous flagella and the ability to form spore (Thakur *et al.,* 2022). It has ability to form a tough protective endospore and resistance to extreme conditions (Zhou *et al.*, 2014). *Bacillus* species are known for their broad-spectrum antimicrobial activity and have gained significant interest as bio-control agents in agriculture (Nirmalkar *et al.,* 2018). Now a day's use of new chemicals for crop management is also governing the growth and performance of bioagents. Having a tolerant strain with all desirable characters, its multiplication and application is a limiting factor for use of bio-control agents (Nirmalkar *et al*., 2020).

The combination of biological control agents with fungicides would result in disease suppression that is comparable to that obtained with more fungicide use (Sharma *et al.,* 2021). With this literature as background, studies were carried out to find the compatibility test of commonly used nineteen fungicides with *Bacillus subtilis* (effective strain BS9) at different concentrations i.e. 100, 500, 1000, 1500 and 2000 ppm using poison food technique under *in vitro* condition.

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# MATERIALS AND METHODS

**Poisoned food technique**

The compatibility of commonly used fungicides that are usually applied for disease management was tested against most effective isolates BS9 following poison food technique. A total of different concentrations (100, 500, 1000, 1500 and 2000 ppm) of each fungicide were taken into study. Fungicides like Hexaconazole 50% SC, Carbendazim 75% WP, Mancozeb 75 %WP, Azoxystrobin 25 % SC, Propiconzole 10.7 % + Tricyclazole 34.2 % SE, Azoxystrobin 18.2% + Difenoconazole 11.4% SC, Tebuconazole 50%+ Trifluoxystrobin 25% WG, Carbendazim 12%+ Mancozeb 63 % WP, Chlorothaonil 75% WP, Trifloxystrobin 25% WG, Difenoconazole 25% EC, Picoxystrobin 7.05% + Propiconazole 11.7 % SC, Fosetyl Al 80 %WP, Copper oxychloride 50% WP, Cyproconazole 800 WG, Azoxystrobin 7.1% + Propiconazole 11.9% SE, Pyroclostrobin 20% WG, Fluxapyroxad 6.25% + Epoxiconazole 6.25% EC and Azoxystrobin 18.2% +Cyproconazole 7.3% W/V were tested. First, stock solutions of fungicides were prepared and then different concentrations of each fungicide were prepared simply by pipetting the required volume from the stock and adding to the sterilized nutrient agar (NA) medium just before pouring to petri dishes. The pour plate method was used to inoculate with the freshly cultured *Bacillus* strains on fungicides amended NA plates and incubated at 28±2$^0$C for 48 hours. Nutrient agar plates without any fungicide were kept as control plates. Three replications were maintained for every concentration of individual fungicide.

Estimation of total bacterial count = No. of CFUs × Reciprocal of dilution factor

**Observations recorded-** CFU's were observed after 72 hours

# RESULTS AND DISCUSSION

The data regarding compatibility test of commonly used nineteen fungicides with *Bacillus subtilis* (effective strain BS9) at different concentrations i.e. 100, 500, 1000, 1500 and 2000 ppm using poison food technique under *in vitro* condition are given below.

**100 ppm**

Maximum colony forming units (CFU ml$^{-1}$) recorded at 100 ppm concentrations after 72 hours of incubation in most of the fungicides except Hexaconazole 50% SC, Mancozeb 75% WP, Carbendazim 12% + Mancozeb 63%, Chlorothaonil 75% WP, Picoxystrobin 7.05% + Propiconazole 11.71% SC and Copper oxychloride 50% WP. However, fungicides i.e. Fosetyl Al 80% WP (181.0× 10$^8$ CFU ml$^{-1}$) followed by Propiconazole 10.7% + Tricyclazole 34.2% SE (175.0× 10$^8$ CFU ml$^{-1}$), Azoxystrobin 18.2% + Difenoconazole @11.4% SC (169.0× 10$^8$ CFUs/ml), Azoxystrobin 18.2% +Cyproconazole 7.3%W/V (162.0×10$^8$ CFU ml$^{-1}$), Azoxystrobin 25 % SC (161.0×10$^8$ CFU ml$^{-1}$) and Difenoconazole 25 % EC (155.0×10$^8$ CFU ml$^{-1}$) were either non inhibitory of less inhibitory to the growth of *Bacillus subtilis* and found highly compatible showing higher number of colony forming units (CFU's) as compared to other fungicides.

**500 ppm**

Maximum colony forming units (CFU ml$^{-1}$) recorded at 500 ppm concentrations after 72 hours of incubation in most of the fungicides i.e. Fosetyl Al 80 %WP (180.0× 10$^8$ CFU ml$^{-1}$) followed by Propiconzole 10.7 % + Tricyclazole 34.2 % SE (175.0× 10$^8$ CFU ml$^{-1}$), Azoxystrobin 18.2% + Difenoconazole 11.4% SC (165.0× 10$^8$ CFU ml$^{-1}$), Azoxystrobin 25 % SC (160.0× 10$^8$ CFU ml$^{-1}$), Difenoconazole 25% EC (148.0× 10$^8$ CFU ml$^{-1}$) and Trifloxystrobin 25% WG (146.0× 10$^8$ CFU ml$^{-1}$) and these were either non inhibitory or less inhibitory to the growth of *Bacillus subtilis* and found highly compatible showing higher number of colony forming units (CFU's) as compared to other fungicides. Whereas, fungicides i.e. Carbendazim 75 WP (137.0× 10$^8$ CFU ml$^{-1}$), Tebuconazole 50%+ Trifluoxystrobin 25% WG (132.0× 10$^8$ CFU ml$^{-1}$) and Azoxystrobin 18.2% + Cyproconazole 7.3%W/V (128.0×10$^8$ CFU ml$^{-1}$) showing moderate inhibitory effect on the growth of *Bacillus subtilis*. While, fungicides i.e. Cyproconazole 800 %WG (50.0× 10$^8$ CFU ml$^{-1}$), Pyroclostrobin 20%WG (91.2× 10$^8$ CFU ml$^{-1}$) and Fluxapyroxad 6.25% + Epoxiconazole 6.25% EC (95.6× 10$^8$ CFU ml$^{-1}$) were least compatible with *Bacillus subtilis* over control (200.0×10$^8$) CFU ml$^{-1}$.

**1000 ppm**

Maximum colony forming units (CFU ml$^{-1}$) recorded at 1000 ppm concentrations after 72 hours of incubation in most of the fungicides i.e. Trifluoxystrobin 25% WG (140.0× 10$^8$ CFU ml$^{-1}$) followed by Azoxystrobin 18.2% + Cyproconazole 7.3%W/V (128.0× 108 CFU ml$^{-1}$), Trifloxystrobin 25% WG (127.0× 10$^8$ CFU ml$^{-1}$) and Carbendazim 75 WP (123.0× 10$^8$ CFU ml$^{-1}$) were either non inhibitory or less inhibitory to the growth of *Bacillus subtilis* and found highly compatible showing higher number of colony forming units (CFU's) as compared to other fungicides. While, fungicides i.e. Fluxapyroxad 6.25% + Epoxiconazole 6.25% EC (22.6× 10$^8$ CFU ml$^{-1}$), Difenoconazole 25% EC (42.6× 10$^8$ CFU ml$^{-1}$) and Azoxystrobin 7.1% + Propiconazole 11.9% SE (81.3 × 10$^8$ CFU ml$^{-1}$) were least compatible with *Bacillus subtilis* over control (179.0 ×10$^8$) CFU ml$^{-1}$.

**1500 ppm**

Maximum colony forming units (CFU ml$^{-1}$) recorded at 1500 ppm concentrations after 72 hours of incubation in most of the fungicides i.e. Azoxystrobin 18.2% + Difenoconazole 11.4% SC (125.0× 10$^8$ CFU ml$^{-1}$) followed by Trifloxystrobin 25% WG (124.0× 10$^8$ CFU ml$^{-1}$) and Azoxystrobin 25 % SC (117.0× 10$^8$ CFU ml$^{-1}$) and these were either non inhibitory of less inhibitory for the growth of *Bacillus subtilis* and found highly compatible showing higher number of colony forming units (CFU's) as compared to other fungicides. Whereas, fungicides i.e. Fosetyl Al 80 %WP (113.0× 10$^8$ CFU ml$^{-1}$) and Azoxystrobin 18.2% + Cyproconazole 7.3%W/V (110.0× 10$^8$ CFU ml$^{-1}$), Carbendazim 75 WP (106.0× 10$^8$ CFU ml$^{-1}$) showed moderate inhibitory effect on the growth of *Bacillus subtilis*. While, fungicides i.e. Fluxapyroxad 6.25% + Epoxiconazole 6.25% EC (25.3× $10^8$ CFU $ml^{-1}$), Azoxystrobin 7.1% + propiconazole 11.9% SE (27.0 × $10^8$ CFU $ml^{-1}$) and Pyroclostrobin 20% WG (29.3× $10^8$ CFU $ml^{-1}$) were least compatible with *Bacillus subtilis* over control (183.0×$10^8$ CFU $ml^{-1}$).

**2000 ppm**

Maximum colony forming units (CFU $ml^{-1}$) recorded at 2000 ppm concentrations after 72 hours of incubation in most of the fungicides i.e. Azoxystrobin 25 % W/W (130.0× $10^8$ CFU $ml^{-1}$) followed by Fosetyl Al 80 %WP (128.0× $10^8$ CFU $ml^{-1}$) and Carbendazim 75 WP (122.0× $10^8$ CFU $ml^{-1}$) and these were either non inhibitory or less inhibitory for the growth of *Bacillus subtilis* and found highly compatible showing higher number of colony forming unit (CFU's) as compared to other fungicides. Whereas, fungicides i.e. Azoxystrobin 18.2% +Cyproconazole 7.3%W/V (107.0× $10^8$ CFU $ml^{-1}$), Trifloxystrobin 25% WG (102.0× $10^8$ CFU $ml^{-1}$) and Azoxystrobin 18.2% + Difenoconazole 11.4% SC (92.0× $10^8$ CFU $ml^{-1}$) showed moderate inhibitory effect on the growth of *Bacillus subtilis*. While, fungicides Propiconzole 10.7 % + Tricyclazole 34.2 % SE (75.3× $10^8$ CFU $ml^{-1}$) and Tebuconazole 50%+ Trifluoxystrobin 25% WG (86.3× $10^8$ CFU $ml^{-1}$) were found least compatible with *Bacillus subtilis* over control (181.0 × $10^8$ CFU $ml^{-1}$).

Colony forming units (CFU's) recorded from different concentrations of fungicides indicates that most of the fungicides Carbendazim 50% WP, Azoxystrobin 25% SC, Propiconazole 10.7% + Tricyclazole 34.2% SE, Azoxystrobin 18.2% + Difenoconazole 11.4% SC, Tebuconazole 50% + Trifluoxystrobin 25% WG, Trifloxystrobin 25% WG, Fosetyl Al 80% WP and Azoxystrobin 18.2% +Cyproconazole 7.3%W/V were observed to be compatible with *Bacillus subtilis* and recorded higher number of colony forming units (CFU's). Whereas, Azoxystrobin 7.1% + Propiconazole 11.9% SE, Fluxapyroxad 6.25 %+ Epoxiconazole 6.25% SC and Pyroclostrobin 20% WG were found to be less compatible with *Bacillus subitilis*. Hexaconazole 50% SC, Mancozeb 75% WP, Carbendazim 12% + Mancozeb 63%, Chlorothaonil 75% WP, Picoxystrobin 7.05% + Propiconazole 11.71% SC and Copper oxychloride 50% WP were completely inhibitory even at 100 ppm concentration. Moreover, Azoxystrobin 25%W/W followed by Fosetyl Al 80%WP and Carbendazim 75%WP were found to be highly compatible with *Bacillus subtilis* and showed higher number of CFU's. Vijaykrishna *et al.* (2011) studied the compatibility of *Bacillus subtilis* strain MBI 600 with various fungicides and observed that the strain showed good compatibility with Carbendazim and Azoxystrobin at a lower concentration of 500 ppm. Similarly, Devi and Prakasam (2013) also reported the compatibility nature of Azoxystrobin 25 SC with *Pseudomonas fluorescens* and *Bacillus subtilis* at 50, 100 and 250 ppm concentrations.

**Table 1. Compatibility of *Bacillus* spp. with different fungicides**

| | Treatments | 100 ppm | 500 ppm | 1000 ppm | 1500 ppm | 2000 ppm |
|---|---|---|---|---|---|---|
| | | **72 hours** | **72 hours** | **72 hours** | **72 hours** | **72 hours** |
| $T_1$ | Hexaconazole 50% SC | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| $T_2$ | Carbendazim 75 WP | 138.0 ×$10^8$ | 137.0× $10^8$ | 123.0× $10^8$ | 106.0× $10^8$ | 122.0× $10^8$ |
| $T_3$ | Mancozeb 75 WP | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| $T_4$ | Azoxystrobin 25 % SC | 161.0× $10^8$ | 160.0× $10^8$ | 113.0× $10^8$ | 117.0× $10^8$ | 130.0× $10^8$ |
| $T_5$ | Propiconzole 10.7 % + Tricyclazole 34.2 % SE | 175.0× $10^8$ | 175.0× $10^8$ | 111.0× $10^8$ | 95.0× $10^8$ | 75.3× $10^8$ |
| $T_6$ | Azoxystrobin 18.2% + Difenoconazole 11.4% SC | 169.0× $10^8$ | 165.0× $10^8$ | 119.0× $10^8$ | 125.0× $10^8$ | 92.0× $10^8$ |
| $T_7$ | Tebuconazole 50%+ Trifluoxystrobin 25% WG | 145.0× $10^8$ | 132.0× $10^8$ | 140.0× $10^8$ | 103.0× $10^8$ | 86.3× $10^8$ |
| $T_8$ | Carbendazim 12%+ Mancozeb 63 % WP | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| $T_9$ | Chlorothaonil 75% WP | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| $T_{10}$ | Trifloxystrobin 25% WG | 153.0× $10^8$ | 146.0× $10^8$ | 127.0× $10^8$ | 124.0× $10^8$ | 102.0× $10^8$ |
| $T_{11}$ | Difenoconazole 25% EC | 155.0× $10^8$ | 148.0× $10^8$ | 42.6× $10^8$ | 0.0 | 0.0 |
| $T_{12}$ | Picoxystrobin 7.05% + Propiconazole 11.7 % SC | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| $T_{13}$ | Fosetyl Al 80 %WP | 181.0× $10^8$ | 180.0× $10^8$ | 115.0× $10^8$ | 113.0× $10^8$ | 128.0× $10^8$ |
| $T_{14}$ | Copper oxychloride 50% WP | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| $T_{15}$ | Cyproconazole 800 WG | 148.0× $10^8$ | 50.0× $10^8$ | 0.0 | 0.0 | 0.0 |
| $T_{16}$ | Azoxystrobin 7.1% + propiconazole 11.9% SE | 140.0× $10^8$ | 123.0× $10^8$ | 81.3× $10^8$ | 27.0× $10^8$ | 0.0 |
| $T_{17}$ | Pyroclostrobin 20% WG | 110.0× $10^8$ | 91.2× $10^8$ | 109.0× $10^8$ | 29.3× $10^8$ | 0.0 |
| $T_{18}$ | Fluxapyroxad 6.25% + Epoxiconazole 6.25% EC | 100.0× $10^8$ | 95.6× $10^8$ | 22.6× $10^8$ | 25.3× $10^8$ | 0.0 |
| $T_{19}$ | Azoxystrobin 18.2% + Cyproconazole 7.3% W/V | 162.0× $10^8$ | 128.0× $10^8$ | 128.0× $10^8$ | 110.0× $10^8$ | 107.0× $10^8$ |
| $T_{20}$ | Control | 199.0× $10^8$ | 200.0× $10^8$ | 179.0× $10^8$ | 183.0× $10^8$ | 181.0× $10^8$ |

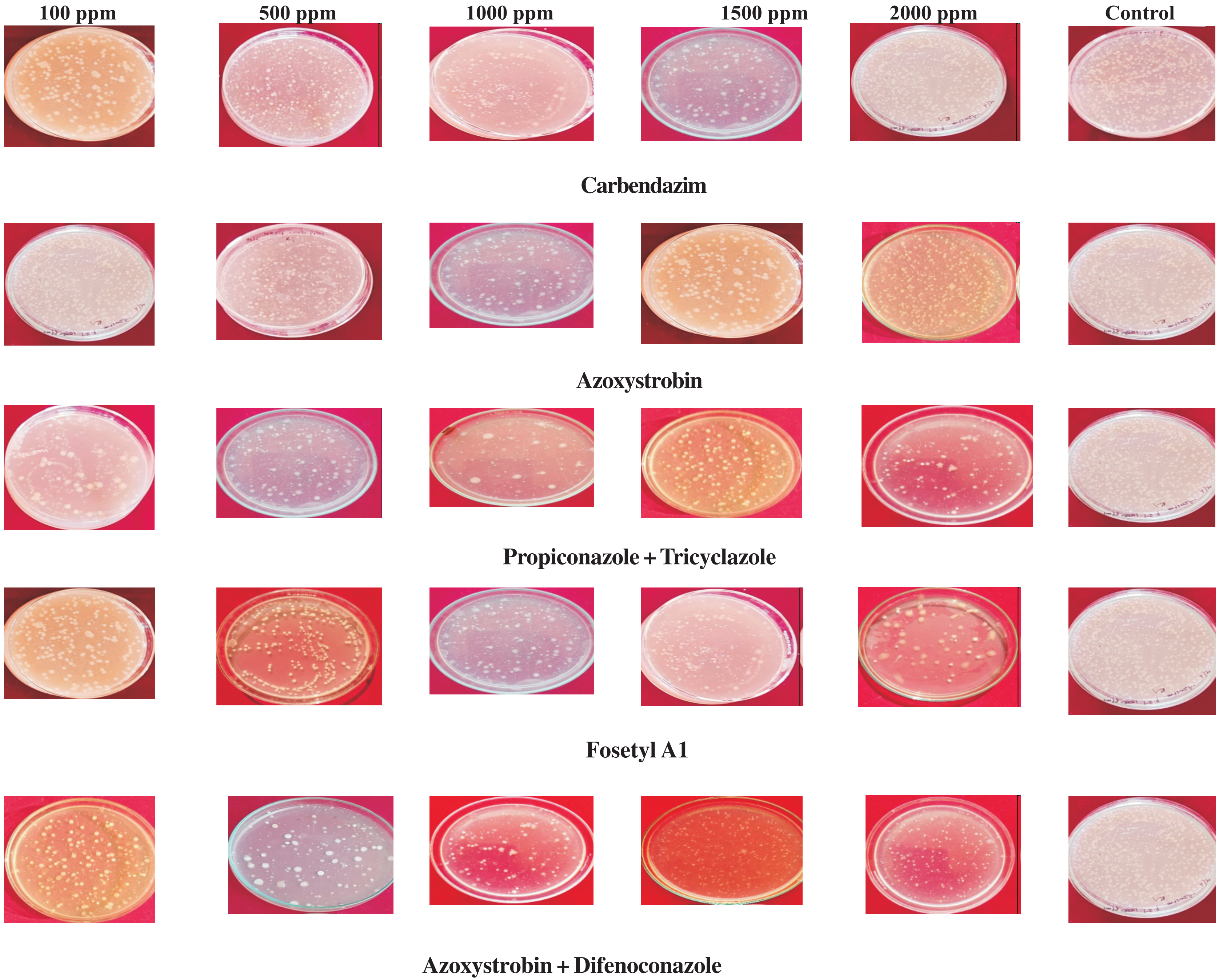


**Figure 1 (a). Compatibility of *Bacillus* spp. with different fungicides CFU's after 72 hours**

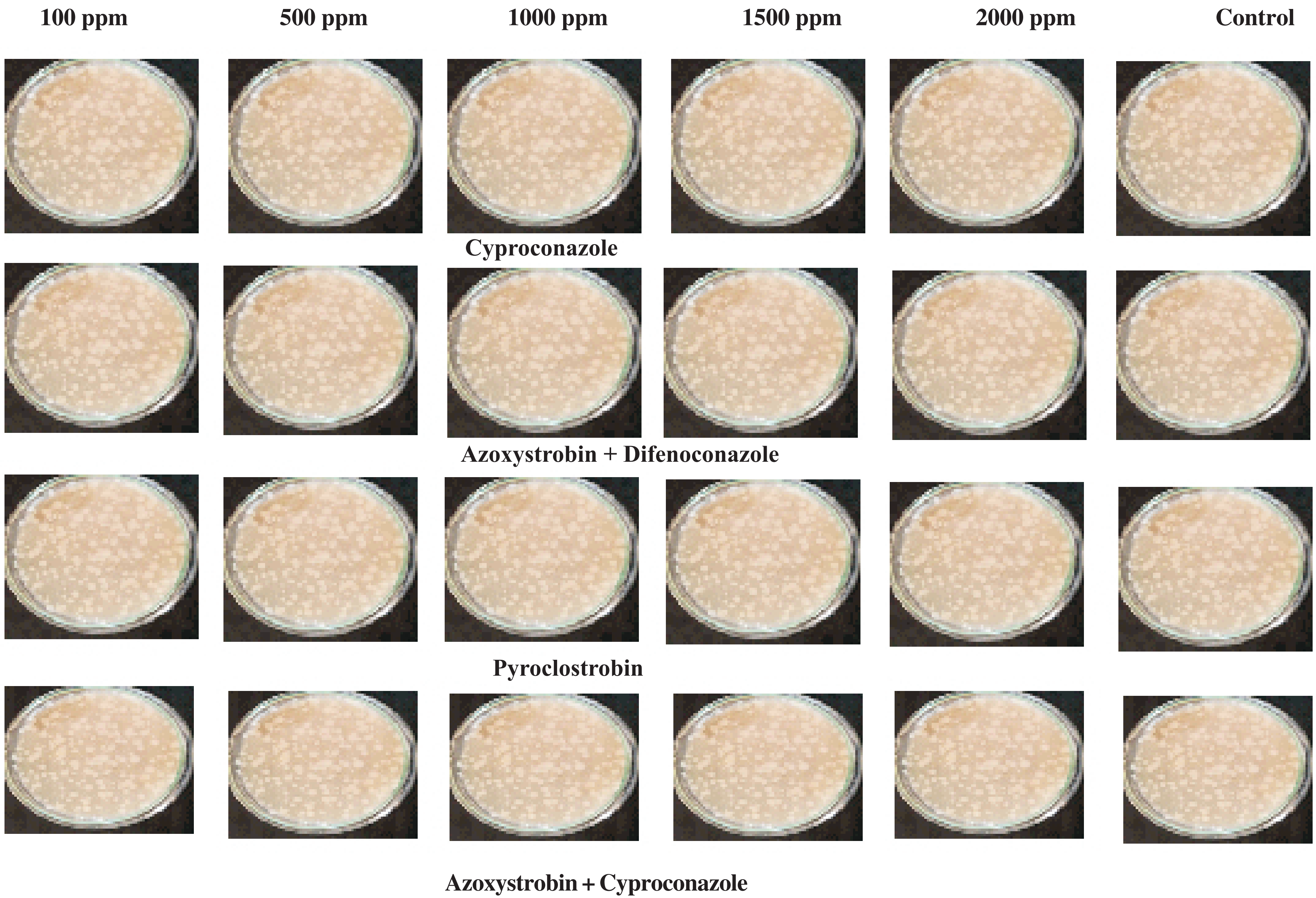


**Figure 1 (b). Compatibility of *Bacillus* spp. with different fungicides CFU's after 72 hours**

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