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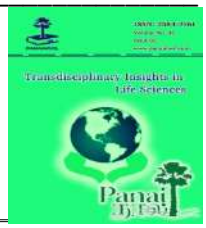

RESEARCH ARTICLE

Compatibility studies on *Bacillus subtilis* treated with Agrochemicals and Seaweed fertilizers

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ABSTRACT

The present study was conducted to find out compatibility of fungicides with different concentration such as Monocrotophos, Mancozeb, Tilt and Thiovit was tested on *Bacillus subtilis*. Among all the fungicides tested were enhanced the growth of *Bacillus subtilis* at all concentrations where as other fungicides like Monocrotophos, mancozeb, Tilt and Thiovit was compatible. Because it does not inhibits the growth of *Bacillus subtilis* when compared to Control (Sea weeds) plate. Among the all four chemical fungicides tested, exhibits better growth of *Bacillus subtilis* when compared to the Control (Sea weeds) plate. So the fungicides enhance the growth of *Bacillus subtilis* and all the fungicides were increased the growth of *Bacillus subtilis*. The present study concluded that mostly chemical fungicides were not compatible with *Bacillus subtilis*. From our results revealed that all of the fungicides tested were inhibit the growth of *Bacillus subtilis* at all the concentrations. Henc, the study suggested that the few of agrochemicals were also not compatible with *Bacillus subtilis*.

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Introduction

Soil application of bio-inoculants, insecticides, organic amendments, and mineral fertilizers is a regularly used way to enhance crop yield and economic return (Mahmud et al., 2021). Despite the implementation of contemporary agricultural practices, illnesses lead to a loss of more than 10% of total crop production, even with the use of existing control methods such as seaweeds. Fungi can inflict significant harm in agriculture, leading to substantial reductions in output, quality, and profit (El Boukhari et al., 2020; Toledo et al., 2023). Fungicides safeguard agricultural products from deterioration and contamination by hazardous fungal toxins (Zadravec et al., 2022). Commercial cultivation of numerous crops, particularly fruits and vegetables, in humid climates necessitates the application of fungicides in disease control strategies. Typically, fungicides work by impeding the energy metabolism, obstructing biosynthesis, or modifying the cell membranes of the fungus (Gikas et al., 2022). There is less information regarding the impact of fungicides on the indigenous microbial community in soils, particularly Plant Growth Promoting Rhizobacteria

(PGPR) (Khoso et al., 2024; Sabaridasan, 2012). Some chemical components of fungicides can disrupt or hinder the physiological or metabolic functions of plants, impede electron transport reactions in chloroplasts, and decrease plant development (Shahid et al., 2018). Sea weed biological control chemicals protect roots by inhibiting phytopathogenic fungi and bacteria through antagonism (Vicente et al., 2023). Rhizosphere bacteria are effective in managing soil-borne plant diseases such as weeds (Saeed et al., 2021). Previous reports have indicated that many bacteria, including *Bacillus subtilis*, Bacillus sp., Serratia sp., and Arthobacter sp., have the ability to promote plant growth (Chhetri et al., 2022; De Mandal et al., 2018; Hashem et al., 2019).

Bacillus subtilis is a rhizobacterium that promotes plant development. Specific strains of *Bacillus subtilis* synthesize secondary compounds that are harmful to plant-pathogenic fungi (Jan et al., 2023). Enhancing the production of antifungal chemicals in bacteria can help them reduce plant infections and improve their ecological competitiveness in the rhizosphere (Ayaz et al., 2023). Particular strains of *Bacillus subtilis* and *Pseudomonas putida* offer biological control of fungal plant diseases and harmful rhizobacteria, resulting in enhanced growth responses that go beyond just disease improvement (Bonaterra et al., 2022).

Soil microorganisms are affected by alterations in their soil environment (J. Li et al., 2022), and research has demonstrated that the microbial community shifts during fertilization (Dincă et al., 2022). Fertilizer can enhance the growth of microbial populations by providing nutrients and may influence the makeup of specific microbial communities in the soil (Zhang et al., 2022).

Microbial biomass and enzyme activity are now acknowledged as early markers of soil stress or changes in productivity. Moreover, there is much evidence indicating that they can be utilized to assess the impact of management and land use on soils (Q. Qu et al., 2023; R. Qu et al., 2023). The study aimed to evaluate the effects of pesticides and seaweed fertilizers on the beneficial soil microbe *Bacillus subtilis*.

Materials and Methods

Collection and isolation of samples

The soil sample was collected from the paddy field in the current investigation. The material underwent dilution using the conventional serial dilution approach to promote bacterial growth. After the incubation period was finished, the colonies were examined. The detected colonies were plated again to isolate single colonies using the streaking method. The morphology of isolated colonies was identified using the Gram staining method. Gram-positive results in a purple or blue color, while Gram-negative results in a pink or red color. The genus confirmation was conducted using biochemical tests in the laboratory to identify specific beneficial organisms. The selected media were ultimately utilized for species confirmation and identification (Wilson et al., 2017).

(T1) Mancozep

It controls numerous fungal diseases such as blight, leaf spot, rust, downy mildew, scab, and various other diseases. This fungicide is commonly used to control infections in potatoes, tomatoes, cucurbits, beets, berries, and rust on various cereal crops, vegetables, and ornamental plants such as roses, carnations, beans, apples, and plums. It is utilized for foliar application and seed treatment in various agricultural and horticultural crops. It is non-phytotoxic when taken as recommended. This fungicide is quite compatible with the majority of commonly used fungicides and insecticides.

(T2) Propinazole

This is a systemic foliar fungicide that has both protective and curative effects. It controls illnesses caused by *Erysiphe graminis, Puccinia spp., Rhynchosporium secalis,* and *Septoria spp*. As well as in *Rhizoctonia solani* and *Helminthosporium oryzae* in rice; Cercospora in groundnuts; Monilinia and Sphaerotheca in stone fruits; and Helminthosporium in maize. It is non-phytotoxic when used correctly and can be used alongside other fungicides.

(T3) Thiovit (Sulphur)

Non-systemic fungicides and acaricides that act protectively. It controls scab on apples, pears, and peaches; powdery mildews on various crops such as fruit vines, beets, cereals, ornamentals, cucumbers, vegetables, and forestry; and acarinosis of vines. Phytotoxic to cucurbits, rasp berries, and specific "sulphur-shy" types of various crops. Avoid mixing with oil, as it may cause phytotoxicity.

(T4) Monocrotophos

Monocrotophos is an organophosphate pesticide. It is highly toxic to birds and humans, leading to its prohibition in the U.S., the E.U., and several other nations; yet, it remains accessible in India. It is mostly utilized in agriculture as an inexpensive pesticide. However, it is also commonly utilized as a method for suicide. Nevertheless, this herbicide has wide-ranging effects on humans and other creatures. Toxicity effects have been observed in terms of cardiotoxicity and acute effects on the public environment.

Preparation of media

The glassware's and other requisite materials were cleaned and sterilized before being used for the preparation media. The LB media contains of Peptone (10g / 1000ml), Yeast Extract (5g / 1000ml), and Sodium Chloride (5g / 1000ml). These are weighted using the electric balance and mixed there components with sterile distilled water before making the final volume to 1000ml. The media is sterilized at 15lbs for 15min and allowed to cool before the use. The medium is dispensed into 5 flasks and used as a stock media. Each 100ml media was mixed individually with fungicides in different concentration (0ppm, 1000ppm, 1500ppm, 2000ppm, 2500ppm) and used for testing their effect on *Bacillus subtilis*.

Culture and maintenance of Bacillus subtilis

Bacillus subtilis was kept and maintenance active at room temperature in liquid LB medium for use in several experiments in this work (Dervaux et al., 2014).

Serial dilution preparation

9 ml of sterile distilled water was evenly distributed into sterile test tubes under aseptic conditions (Di et al., 2023). 1 ml of actively growing *Bacillus subtilis* from the stock culture is mixed with 9 ml of sterile distilled water to create a 10^{-1} dilution. 1 ml of the sample is combined with 9 ml of distilled water to create a 10^{-2} dilution. A serial dilution was performed up to a 10^{-10} concentration.

Pour plating

Pour plating was performed on sterile petri plates in aseptic conditions. *Bacillus subtilis* was cultured in two distinct dilutions of 10^{-9} and 10^{-10}. Approximately 1 ml of culture from the previous two dilutions was transferred into sterile Petri plates. Once the medium with different amounts of fungicides and bactericides was warm, it was added to the *Bacillus subtilis* that had already been spread out. The mixture was then gently stirred to make sure that the fungicides and bactericides were spread out evenly (Sanders, 2012). The cultures were kept at ambient temperature for 48 hours before being observed. Colony counting data was collected and tabulated for study.

Results and Discussion

Chemical fungicides such as Monocrotophs, Mancozeb, Tilt, and Thiovit were tested for compatibility with seaweeds as a control for *Bacillus subtilis* in the study.

Impact of monocrotophos on the development of Bacillus subtilis after 48 hours of incubation

Bacillus subtilis was exposed to several concentrations of the antibiotic Monocrotophos (500 ppm, 1500 ppm, 1000 ppm, and 2000 ppm). No growth was observed in the 10^{-9} and 10^{-10} dilutions after 48 hours of incubation. The control (seaweeds) plate displayed cell concentrations of 25×10^{-9} cells/ml and 65×10^{-10} cells/ml. The study found that the antibiotic monocrotophos, at various concentrations, resulted in 100% inhibition of *Bacillus subtilis* growth (Table 1).

Table 1. Effect of Monocrotophos on *Bacillus subtilis* after 48 hours incubation

Monocrotophos (ppm)	Colony forming Unit / ml							
	10^{-9} dilution			Enhance/Inhibit of growth	10^{-10} dilution			Enhance/Inhibit of growth
	R1	R2	Mean		R1	R2	Mean	
Control (Sea weeds)	130	120	125	0%	60	70	65	0%
500	-	-	-	-100%	-	-	-	-100%
1000	-	-	-	-100%	-	-	-	-100%
1500	-	-	-	-100%	-	-	-	-100%
2000	-	-	-	-100%	-	-	-	-100%

Impact of Mancozeb on Bacillus subtilis growth after 48 hours of incubation

Bacillus subtilis was exposed to varying concentrations of the antibiotic Mancozeb: 500 ppm, 1500 ppm, 1000 ppm, and 2000 ppm. No growth was observed in the 10^{-9} and 10^{-10} dilutions after 48 hours of incubation. The control (sea weeds) plate displayed cell concentrations of 100×10^{-9} cells/ml and 65×10^{-10} cells/ml. The study indicated that the antibiotic Mancozeb, at various concentrations, exhibited complete suppression of *Bacillus subtilis* growth (Table 2).

Impact of Tilit on the development of Bacillus subtilis following 48 hours of incubation

Bacillus subtilis was exposed to varying concentrations of fungicide. The growth was measured at 25×10^{-9} cells/ml and 10×10^{-10} cells/ml in a 1000 ppm concentration. Cell concentrations were measured at 45 x 10-9 cells/ml and 25×10^{-10} cells/ml, while at a concentration of 2000 ppm, the concentrations were 55×10^{-9} cells/ml and 25×10^{-10} cells/ml. At a dosage of 2500 ppm, there were 70×10^{-9} cells/ml and 45×10^{-10} cells/ml after 48 hours of incubation. Therefore, it was determined that as the concentration of fungicide increased, the growth of the bacteria reduced (Table 3).

Table 2. Effect of Mancozeb on *Bacillus subtilis* after 48 hours incubation

Mancozeb (ppm)	Colony forming Unit / ml							
	10^{-9} dilution			Enhance/Inhibit of growth	10^{-10} dilution			Enhance/Inhibit of growth
	R1	R2	Mean		R1	R2	Mean	
Control (Sea weeds)	90	110	100	0%	90	110	100	0%
500	-	-	-	-100%	-	-	-	-100%
1000	-	-	-	-100%	-	-	-	-100%
1500	-	-	-	-100%	-	-	-	-100%
2000	-	-	-	-100%	-	-	-	-100%

Table 3. Effect of Tilt on *Bacillus subtilis* after 48 hours incubation

Tilt (ppm)	Colony forming Unit / ml							
	10^{-9} dilution			Enhance/Inhibit of growth	10^{-10} dilution			Enhance/Inhibit of growth
	R1	R2	Mean		R1	R2	Mean	
Control (Sea weeds)	210	180	195	0%	90	110	100	0%
500	70	80	75	-61%	40	40	40	-60%
1000	60	50	55	-71%	10	20	15	-85%
1500	30	40	35	-82%	20	0	10	-90%
2000	10	30	20	-89%	0	10	5	-95%

Impact of thiovit on the proliferation of Bacillus subtilis following 48 hours of incubation

Bacillus subtilis was exposed to varying concentrations of Thiovit (500 ppm, 1000 ppm, 1500 ppm, and 2000 ppm). After 48 hours of incubation, the colony counts were as follows: 500 ppm resulted in 50×10^{-9} cells/ml and 25×10^{-10} cells/ml, while 1000 ppm yielded 20×10^{-9} cells/ml and 2×10^{-10} cells/ml. However, growth was inhibited at 1500 ppm and 2000 ppm concentrations. At 1500 ppm, the cell concentrations are 35×10^{-9} cells/ml and 20×10^{-10} cells/ml. At 2000 ppm, the cell concentrations are 15×10^{-9} cells/ml and 2×10^{-10} cells/ml. The results indicated that varying concentrations of thiovit (500 ppm, 1000 ppm) promoted the growth of *Bacillus subtilis* compared to the control group (sea weeds). Conversely, concentrations of 1500ppm and 2000ppm resulted in the lowest colony counts compared to 500ppm and 1000ppm. Therefore, *Bacillus subtilis* growth was compatible at concentrations below 1000 ppm (Table 4).

Table 4. Effect of Thiovit on *Bacillus subtilis* after 48 hours incubation

Thiovit (ppm)	Colony forming Unit / ml							
	10^{-9} dilution			Enhance/Inhibit of growth	10^{-10} dilution			Enhance/Inhibit of growth
	R1	R2	Mean		R1	R2	Mean	
Control (Sea weeds)	40	40	40	0%	20	30	25	0%
500	40	60	50	25%	30	20	25	25%
1000	50	40	45	12%	20	20	20	0%
1500	30	40	35	-12%	0	10	10	-60%
2000	10	20	15	-60%	2	2	2	-92%

Discussion

Modern agriculture relies on the use of fossil fuel-based inputs such as chemical fertilizers, herbicides, and high-energy-intensive farm equipment to increase efficiency. High-energy input technologies have unquestionably boosted production levels (Woods et al., 2010). Farmers are increasingly worried about the negative impact on soil productivity and environmental quality, which emphasizes their social responsibilities beyond just being agribusiness owners (Muhie, 2022).

In 1965–1966, high-yielding varieties were introduced in the Indian subcontinent in anticipation of a famine outbreak as part of the 'Green Revolution' technology. The high-yielding variety produced an extraordinarily abundant amount of food grains, meeting the demand to feed the increasing population (Swaminathan, 2001). These high-yielding types, although productive, were more vulnerable to pests compared to older varieties. To combat these pests, a significant quantity of chemical insecticides were utilized. This resulted in environmental damage, greater pest resistance, insect resurgence, and higher levels of chemical residues in agricultural products. As the pollutants seeped into water sources, groundwater was contaminated (Dang et al., 2017; Sánchez-Bayo, 2021).

India is expected to produce 250 million tons of agricultural grains by 2020. Between 1965 and 1975, the production of food grains reached 2.48 million tons by utilizing 23.15 lakh million tons of chemical fertilizer and 47091 tons of chemical insecticides during the initial phase of high-yielding variety production (Ansari & Sheereen, 2022). In the past, NPK materials and organic manure and green manure were highly valued. The modernization of agriculture led to a gradual depletion of organic manure, which is a significant factor in determining fertility (Dhaliwal et al., 2023).

The soil contains various important components necessary for plant growth. Reducing the use of organic manure led to the need for supplementary micronutrients in the form of artificial salts. To utilize fertilizers effectively, ensure efficient utilization, and maintain a balanced amount of all important plant nutrients, This also applies to pest control (Köninger et al., 2021; Thapa et al., 2021). The need to attain sustainable food production through eco-friendly nutrition and pest management technology is increasingly recognized in light of this worrying scenario. It is anticipated that biopesticides will substitute a minimum of 10% of the synthetic pesticides in present use (Fenibo et al., 2021; Pathak et al., 2022). For sustainable agriculture, it involves combining chemicals and biological substances in fertilizer delivery systems. However, it merges conventional conservation-focused approaches with contemporary innovations like enhanced seeds (Akhtar et al., 2022; Magnabosco et al., 2023). Modern equipment incorporates weed management systems that integrate nutrient supply with nitrogen-fixing bacteria, phosphorus solubilizers, and other biological agents. The study found a notable variance in how different compounds affect the radial growth inhibition percentage of *Bacillus subtilis* (Gupta et al., 2022). *Bacillus subtilis* thrived on culture media with fungicides at concentrations of up to 1000 ppm. After 48 hours, the radial mycelial growth data of *Bacillus subtilis* at the recommended dosages of 51% fungicides showed a significantly higher growth percentage compared to other treatments (Y. Li et al., 2023; Yu et al., 2021).

It appears that the fungicides did not inhibit the growth of *Bacillus subtilis*. Studies indicate that *Bacillus subtilis* can be safely utilized in integrated pest management for disease control in seaweeds, particularly when used with fungicides that include 51%. However, when combining other fungicides with bactericides, caution must be exercised. One must establish specific intervals when applying *Bacillus subtilis* and other fungicides. The radial mycelial growth (in cm)

showed that agrochemicals do not hinder *Bacillus subtilis*, but all other fungicides do restrict the radial mycelial development.

Previous researchers have noted similar findings, and our analysis validates this. When the pathogenic organism dominates, generating high-pressure conditions, the use of *Bacillus subtilis* may not effectively control the disease in sea weeds. Advocates of the organic-inorganic management strategy suggest using a mix of biological agents and chemical fungicides (Isidori et al., 2021; Lee et al., 2022). This study is significant as it shows the compatibility of *Bacillus subtilis* with fungicides like kavanch, out sore, tilt, and thiovit. It demonstrates that these chemical fungicides can be used in conjunction with *Bacillus subtilis* to control fungal diseases in agricultural crops. Several studies suggest that field evaluations should be conducted to examine the interactions between *Bacillus subtilis* and agrochemicals at the field level.

Conclusion

The study tested the compatibility of fungicides like Monocrotophos, Mancozeb, Tilt, and Thiovit on *Bacillus subtilis*. All tested fungicides enhanced the growth of *Bacillus subtilis* at all concentrations, while other fungicides were compatible. However, none inhibited the growth of *Bacillus subtilis* compared to the control (Sea weeds) plate. The study concluded that most chemical fungicides were not compatible with *Bacillus subtilis*, and a few agrochemicals were also not compatible. The results showed that all tested fungicides inhibited the growth of *Bacillus subtilis* at all concentrations, indicating that agrochemicals are not suitable for treating *Bacillus subtilis*

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Conflict of interest

All authors declare that there is no conflict of interest in this work.

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