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Tolerance of Phosphorus and Potassium Solubilizing Bacteria to Fungicides *In vitro*

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Phosphorus solubilizing bacteria (PSB) and Potassium solubilizing bacteria (KSB) plays a vital role in converting these nutrients into forms that plants can readily absorb, thus enhancing soil fertility and crop yields. The compatibility of phosphorus and potassium solubilizing bacteria with 8 fungicides was tested under laboratory condition. The eight fungicides tested against PSB-1, the Carbendazim 50% WP, Propiconazole 25% EC and Carbendazim 12% + Mancozeb 63% WP were

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found highly compatible with phosphorus solubilizing bacteria and rest of the five fungicides viz., Mancozeb 75% WP, Zineb 75% WP, Metalaxyl 35 % WS, Azoxystrobin 18.2 % + Difenoconazole 11.4 % SC, Azoxystrobin 11% + Tebuconazole 18.3 % were incompatible with the test bacterium. The treatment T8 (Azoxystrobin 11% + Tebuconazole 18.3 %) were found statistically at par at 48 and 72 hrs of incubation at 50% of recommended dose and recommended dosages. the eight fungicides tested against KSB-2 Carbendazim 50% WP, Propiconazole 25% EC and Carbendazim 12% + Mancozeb 63% WP were found highly compatible with phosphorus solubilizing bacteria and rest of the five fungicides viz., Mancozeb 75% WP, Zineb 75% WP, Metalaxyl 35 % WS, Azoxystrobin 18.2 % + Difenoconazole 11.4 % SC, Azoxystrobin 11% + Tebuconazole 18.3 % were incompatible bacterium with the test bacterium. The treatment T8 (Azoxystrobin 11% + Tebuconazole 18.3% SC) and treatment T1 (Mancozeb 75% WP) were found statistically at par at 48 and 72 hrs of incubation at 50% of recommended dose and recommended dosages.

Keywords: Fungicides; phosphorus solubilizing bacteria; potassium solubilizing bacteria; compatibility.

1. INTRODUCTION

Phosphorus is a significant nutrient that limits growth, it cannot be obtained from a vast atmospheric source like nitrogen can be accessible biologically. Phosphorus is one of the essential components of crop development. It is linked to a number of essential processes and is in charge of numerous aspects of plant growth, including the formation of nodules, cell division and organization, root development, stalk and stem strength, flower and seed formation, crop maturity and plant disease resistance are attributes associated with phosphorus nutrition (Nwanyanwu et al. 2015). Potassium (K) is the third macronutrient that crop plants need. It is important for growth, yield and the activation of several metabolic processes, such as protein synthesis, photosynthesis and enzyme synthesis, in addition to giving plants resistance to diseases (Raghavendra et al. 2018). Phosphorus solubilizing bacteria (PSB) are widely distributed with varying forms and population densities across different soil types, influenced by soil properties such as physical and chemical attributes, organic matter and phosphorus content high proportion of PSB is `concentrated in the rhizosphere, they were metabolically active (khan et al. 2009; Dinkwar et al., 2020). Potassium can be liberated from insoluble minerals by K-solubilizing bacteria. by inhibiting pathogens and enhancing the nutrients and structure of the soil, K solubilizing bacteria (KSB) can have positive effects on plant growth (Goswami et al. 2019). Among essential nutrients, potassium (K) and phosphorus (P) are crucial for plant growth and development. However, a large proportion of these nutrients in soil are present in insoluble forms, making them inaccessible to plants. Phosphorus solubilizing bacteria (PSB) and Potassium solubilizing bacteria (KSB) plays a vital role in converting these nutrients into forms that plants can readily absorb, thus enhancing soil fertility and crop yields. The combined application of KSB and PSB can have synergistic effects on plant growth and soil health. the eight fungicides that are compatible with phosphorus and potassium-solubilizing bacteria.

2. MATERIALS AND METHODS

2.1 Isolation of Phosphate and Potassium Solubilizing Bacteria

From the rhizosphere soil, PSB and KSB were be separated on selective culture media, such as Pikovskaya's medium and Aleksandrov medium, respectively and pure cultures were preserved for further studies. By using the serial dilution approach, PSB and KSB were separated from a rhizosphere soil sample for the intended use. A gram of soil sample were mixed with nine ml of distilled water and given a good shake. The aforesaid one ml solution was again transferred to 9 ml of sterile distilled water to form10^{-2} dilution. Similarly, upto10^{-3}, 10^{-4}, 10^{-5}, $10^{-6,}$ 10^{-7} and 10^{-8}. on Pikovaskaya's agar medium (PVK), which contains insoluble tricalcium phosphate and Aleksandrov agar media, which contains potassium alumina silicate, each of the 0.1 ml dilutions was dispersed. For 7 days incubated at 27-30℃, colonies showing halo zones were picked and purified on Pikovaskaya's (PVK) agar medium and Aleksandrov agar medium for studying the colony morphology.

2.2 *In vitro* compatibility of PSB and KSB with various Fungicides

Various fungicides will be evaluated *In vitro,* each respectively at two different dosages i.e.

recommended field dose @ 50 % of recommended dose and recommended dose, respectively, to access their compatibility with PSB and KSB by employing paper disc method. PSB-1 and KSB-2 isolates were studied for their resistance towards different fungicides. through paper disc method. Stock solutions of test fungicide. were prepared by adding various concentrations of commercial formulation of fungicides. in distilled water. Paper discs (5 mm diameter) were prepared by adding three different concentrations of test fungicides. One ml of individual bacterial culture will be spread on Pikovskaya's agar and Aleksandrow agar plates with sterile glass spreader aseptically. The 5mm disc of Whatman's filter paper No.41 will be soaked in each concentration for 5 minutes. These discs were then kept on sterilized blotter paper to drain out the excess water from the disc. These discs were placed at equidistance on the agar surface. The plates were incubated for 2-3 days at 28 ± 2 ℃ and the diameter of the inhibition zones was measured in mm.

Experimental Details

Design: Completely randomized design (CRD)

Replication: Three

Treatment: Nine

Observations on zone of inhibition will be recorded at 48 and 72 hrs of the incubation to know the effect of various tests of agrochemicals on PSB and KSB. Per cent growth inhibition of test PSB and KSB will be calculated by applying the formula given by Vincent (1947).

$$\text{Per cent inhibition} = \frac{C - T}{C} \times 100$$

Where,

C = Growth of test isolates in control in mm.
T = Growth of test isolates in treatment in mm.

3. RESULTS AND DISCUSSION

The results revealed that eight fungicides evaluated *in vitro,* exhibited varied inhibition zone at 50 % of recommended dose and recommended dose by maintaining three replication which indicated the degree of compatibility of phosphorus and potassium solubilizing bacteria with the test fungicides.

3.1 *In vitro* Compatibility of Fungicides with Phosphorus Solubilizing Bacteria at 48 and 72 hrs

The results present in Table 1 and Plate 1 revealed that all of the eight fungicides tested at various concentrations, exhibited significant differences in the amount of inhibition zone (mm) recorded at 48 and 72 hrs of incubation. Further, the zone of inhibition was found increased steadily with increase in concentrations of the test fungicides.

The data assessed the inhibition zones produced by various fungicides against phosphorus solubilizing bacteria over two incubation periods (48 and 72 hours). At 48 hours, the inhibition zones ranged from 0.00 to 18.13 mm for 50% of the recommended dose and 0.00 to 18.77 mm at the recommended dose, averaging 0.00 to 18.45 mm. By 72 hours, these zones increased to 0.00 to 19.08 mm and 0.00 to 19.56 mm, respectively, with an average of 0.00 to 19.32 mm. Notably, Carbendazim 50% WP, Propiconazole 25% EC, and Carbendazim 12% + Mancozeb 63% WP showed no inhibition, indicating compatibility with the bacteria, while five other fungicides, including Mancozeb and Azoxystrobin mixtures, demonstrated significant inhibition, confirming their sensitivity. The treatment T8 (Azoxystrobin 11% + Tebuconazole 18.3%) displayed comparable results at both doses across the incubation periods.

Thus, the eight fungicides tested, Carbendazim 50% WP, Propiconazole 25% EC and Carbendazim 12% + Mancozeb 63% WP were found highly compatible with phosphorus solubilizing bacteria and rest of the five fungicides viz., Mancozeb 75% WP, Zineb 75% WP, Metalaxyl 35 % WS, Azoxystrobin 18.2 % + Difenoconazole 11.4 % SC, Azoxystrobin 11% + Tebuconazole 18.3 % were incompatible bacterium with the test bacterium. The treatment T_8 (Azoxystrobin 11% + Tebuconazole 18.3 %) were found statistically at par at 48 and 72 hrs of incubation at 50% of recommended dose and recommended dosages.

3.2 *In vitro* Compatibility of Fungicides with Potassium Solubilizing Bacteria at 48 and 72 hrs

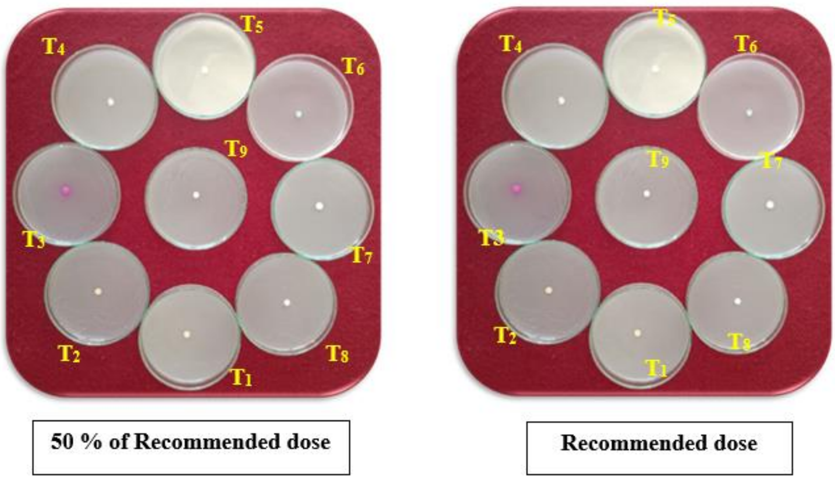
The results in Table 2 and Plate 2 revealed that all of the eight fungicides tested at various concentrations, exhibited significant differences in the amount of inhibition zone (mm) recorded at

48 and 72 hrs of incubation. Further, the zone of inhibition was found to be increased steadily with increase in concentrations of the test fungicides.

The data assessing the efficacy of various fungicides on potassium solubilizing bacteria, the inhibition zones observed at 48 hours of incubation ranged from 0.00 to 20.11 mm for 50% of the recommended doses and 0.00 to 20.88 mm for the full recommended doses, averaging 0.00 to 20.49 mm. By 72 hours, these inhibition zones increased to 0.00-21.37 mm and 0.00-21.93 mm, respectively, with an average of 0.00 to 21.67 mm. Among the eight fungicides tested, Carbendazim 50% WP, Propiconazole 25% EC, and Carbendazim 12% + Mancozeb 3% WP showed no inhibition, indicating high compatibility with the bacteria. Conversely, five fungicides, including Mancozeb 75% WP and various Azoxystrobin combinations, demonstrated significant inhibition, marking them as incompatible. Treatments T8 (Azoxystrobin 11% + Tebuconazole 18.3% SC) and T1 (Mancozeb 75% WP) were statistically similar in their effects at both incubation periods.

List 1. Treatment details

Tr.No.	Treatments	Tr.No.	Treatments
T_1	Mancozeb 75% WP	T_6	Azoxystrobin 18.2% + Difenoconazole 11.4% SC
T_2	Zineb 75% WP	T_7	Carbendazim 12% + Mancozeb 63% WP
T_3	Metalaxyl 35% WS	T_8	Azoxystrobin 11% + Tebuconazole 18.3% SC
T_4	Carbendazim 50% WP	T_9	Control
T_5	Propiconazole 25% EC		



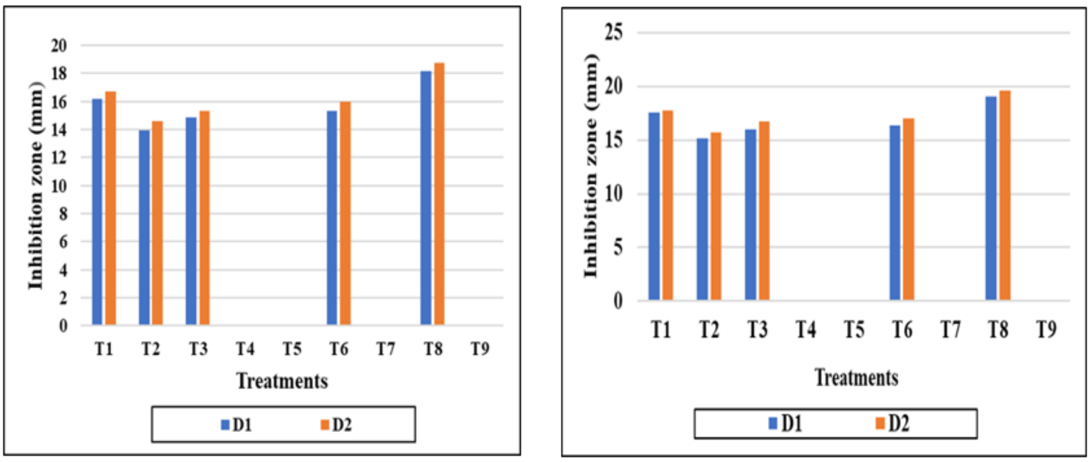


Plate 1. Graph showing inhibition zone for different treatments with fungicides at 48 and 72 hrs

Table 1. *In vitro* compatibility of phosphorus solubilizing bacteria with fungicides at 48 and 72 hrs

Tr. No	Treatments	Inhibition Zone *(mm)at 48 hrs and dosages		Av. Inhibition Zone (mm)	Inhibition Zone *(mm) at 72 hrs and Dosages		Av. Inhibition Zone (mm)
		50% R. D	R D		50% R D	RD	
T1	Mancozeb 75% WP	16.17	16.72	16.44	17.56	17.78	17.67
T2	Zineb 75% WP	13.96	14.58	14.27	15.13	15.74	15.43
T3	Metalaxyl 35 % WS	14.88	15.32	15.10	16.02	16.73	16.37
T4	Carbendazim 50% WP	00.00	00.00	00.00	00.00	00.00	00.00
T5	Propiconazole 25% EC	00.00	00.00	00.00	00.00	00.00	00.00
T6	Azoxystrobin 18.2% + Difenoconazole 11.4 % SC	15.33	15.97	15.65	16.33	16.98	16.65
T7	Carbendazim 12% + Mancozeb 63% WP	00.00	00.00	00.00	00.00	00.00	00.00
T8	Azoxystrobin 11% + Tebuconazole 18.3% SC	18.13	18.77	18.45	19.08	19.56	19.32
T9	Control	00.00	00.00	00.00	00.00	00.00	00.00
S.E. ±		0.39	0.44	-	0.38	0.43	-
C.D.(P=0.01)		1.62	1.79	-	1.58	1.73	-

** Mean of three replications, RD = Recommended dose*

Table 2. *In vitro* compatibility of potassium solubilizing bacteria with fungicides at 48 and 72 hrs

Tr. No.	Treatments	Inhibition Zone*(mm) at 48 hrs and Dosages		Av. Inhibition Zone(mm)	Inhibition Zone *(mm) at 72 hrs and Dosages		Av. Inhibition Zone(mm)
		50% RD	RD		50% RD	RD	
T1	Mancozeb 75% WP	19.50	19.76	19.63	20.11	20.87	20.49
T2	Zineb 75% WP	17.13	17.68	17.40	18.09	18.94	18.51
T3	Metalaxyl 35% WS	15.26	15.81	15.53	16.27	16.89	16.58
T4	Carbendazim 50% WP	00.00	00.00	00.00	00.00	00.00	00.00
T5	Propiconazole 25% EC	00.00	00.00	00.00	00.00	00.00	00.00
T6	Azoxystrobin 18.2% + Difenoconazole 11.4% SC	16.05	16.67	16.36	17.13	17.76	17.44
T7	Carbendazim 12 % + Mancozeb 63% WP	00.00	00.00	00.00	00.00	00.00	00.00
T8	Azoxystrobin 11% + Tebuconazole 18.3% SC	20.11	20.88	20.49	21.37	21.93	21.65
T9	Control	00.00	00.00	00.00	00.00	00.00	00.00
S.E.±		0.41	0.39	-	0.41	0.38	-
C.D. (P=0.01)		1.70	1.60	-	1.68	1.57	-

**Mean of three replications, RD = Recommended dose*

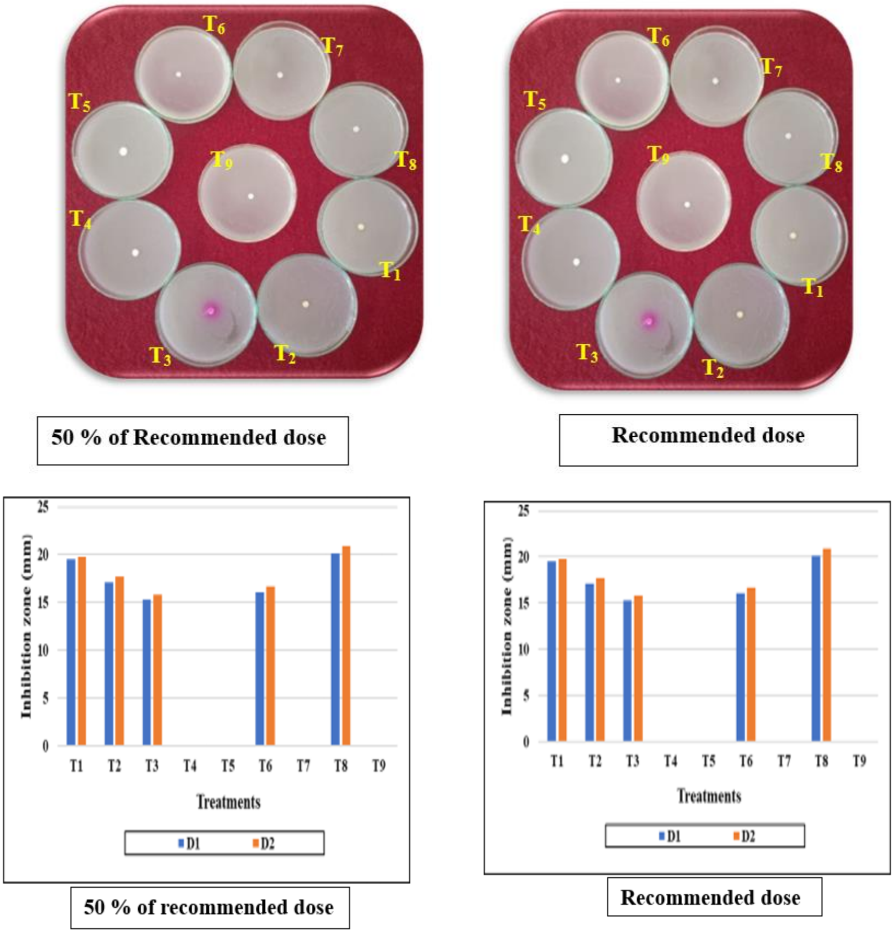


Plate 2. Graph showing inhibition zone for different treatments with solubilizing bacteria with fungicides at 48 and 72 hrs

Found that, fungicides carbendazim 50% WP, Propiconazole 25% EC, Mancozeb 75% WP and Tebuconazole 50% + Trifloxystrobin 25% WG were highly compatible with *Pseudomonas fluorescens*. Purushothaman et al. (2014) studied that bacterial isolates were found to be compatible with Carbendazim and Propiconazole. Vishakha et al. (2019) reported that, among nine fungicides Carbendazim 50% WP, Propiconazole 25% EC, Carbendazim 25% compatible with *Pseudomonas fluorescens*.

4. CONCLUSION

Among the eight fungicides tested against (PSB 1) phosphate solubilizing bacteria and (KSB -2) potassium solubilizing bacteria highly compatible with the Carbendazim 50% WP, Propiconazole 25% EC and Carbendazim 12% + Mancozeb 63% WP and incompatible with and rest of the fungicides viz., Mancozeb 75% WP, Zineb 75% Difenoconazole 11.4 % SC, Azoxystrobin 11% + production. Tebuconazole 18.3 % were incompatible bacterium with the test bacterium. Similar was done by Ashwini et al. (2019) WP, Metalaxyl 35 % WS, Azoxystrobin 18.2 % + reported that, seven fungicides tested the Carbendazim 50 WP%, Propiconazole 25% EC and Tebuconazole 50% + Trifloxystrobin 25% WG were found highly compatible with Phosphate and potassium solubilizing bacteria and rest of four fungicides were found non compatible.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

I hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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