

## **Tolerance of phosphorus and potassium solubilizing bacteria to fungicides in vitro**

### **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 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 % + Difenconazole 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 % + Difenconazole 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

### **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 (Nwanyanwue *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. A 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) play 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.

## **Material and Methods**

### **Isolation of phosphate and potassium solubilizing bacteria**

From the rhizosphere soil, PSB and KSB were 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 was 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 form  $10^{-2}$  dilution. Similarly, up to  $10^{-3}$ ,  $10^{-4}$ ,  $10^{-5}$ ,  $10^{-6}$ ,  $10^{-7}$  and  $10^{-8}$ . On Pikovskaya'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°C, colonies showing halo zones were picked and purified on Pikovskaya's (PVK) agar medium and Aleksandrov agar medium for studying the colony morphology.

### ***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  $28 \pm 2$  °C and the diameter of the inhibition zones was measured in mm.

#### **Experimental details:**

**Design:** Completely randomized design (CRD)

**Replication:** Three

**Treatment:** Nine

#### **List 1: Treatment Details:**

<b>Tr. No.</b>	<b>Treatments</b>	<b>Tr. No.</b>	<b>Treatments</b>
<b>T<sub>1</sub></b>	Mancozeb 75% WP	<b>T<sub>6</sub></b>	Azoxystrobin 18.2% + Difenconazole 11.4% SC
<b>T<sub>2</sub></b>	Zineb 75% WP	<b>T<sub>7</sub></b>	Carbendazim 12% + Mancozeb 63% WP
<b>T<sub>3</sub></b>	Metalaxyl 35% WS	<b>T<sub>8</sub></b>	Azoxystrobin 11% + Tebuconazole 18.3% SC
<b>T<sub>4</sub></b>	Carbendazim 50% WP	<b>T<sub>9</sub></b>	<b>Control</b>
<b>T<sub>5</sub></b>	Propiconazole 25% EC		

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).

C - T

Per cent inhibition = ----- × 100

C

Where, C = Growth of test isolates in control in mm.

T = Growth of test isolates in treatment in mm.

### **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.

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

The results present in Table 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

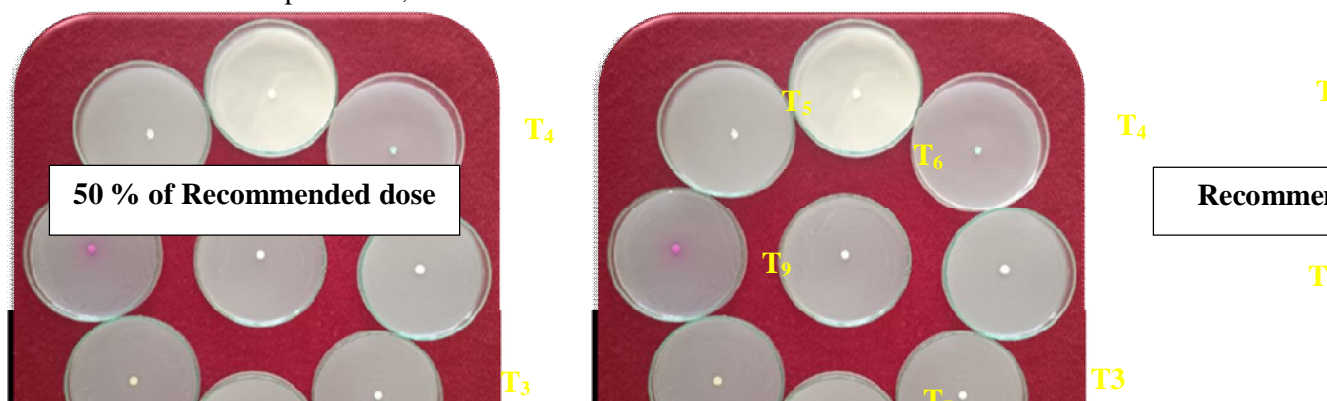
phosphorus solubilizing bacteria and rest of the five fungicides viz., Mancozeb 75% WP, Zineb 75% WP, Metalaxyl 35 % WS, Azoxystrobin 18.2 % + Difenconazole 11.4 % SC, Azoxystrobin 11% + Tebuconazole 18.3 % were incompatible bacterium with the test bacterium. The treatment T<sub>8</sub> (Azoxystrobin 11% + Tebuconazole 18.3 %) were found statistically at par at 48 and 72 hrs of incubation at 50% of recommended dose and recommended dosages.

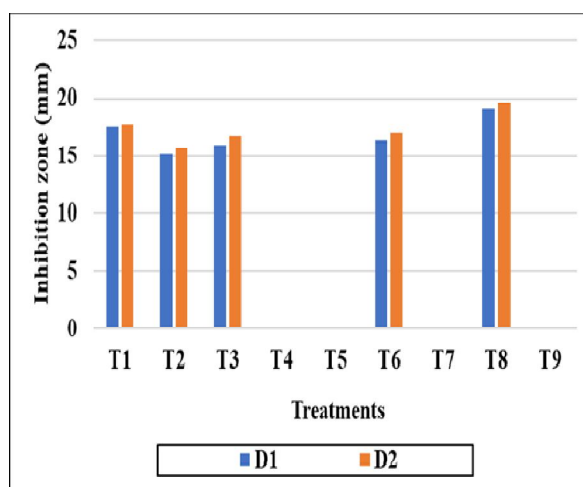
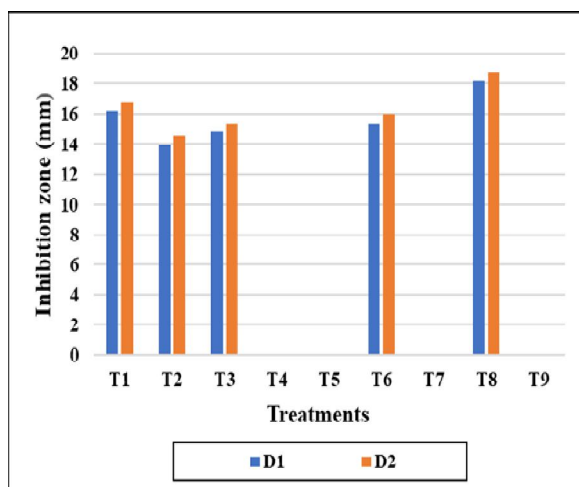
**Table 1: *In vitro* compatibility of phosphorus solubilizing bacteria with fungicides at 48 and 72 hrs**

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	RD		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% + Difenconazole 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





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

**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.

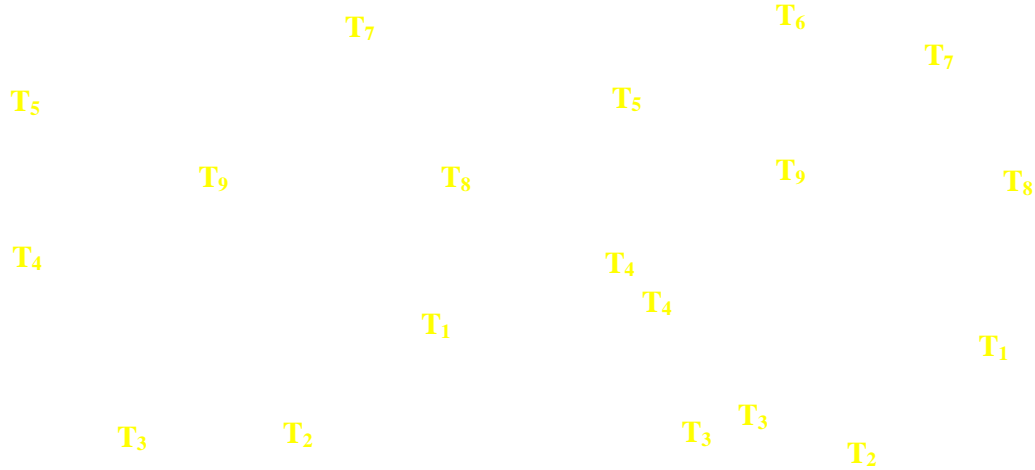
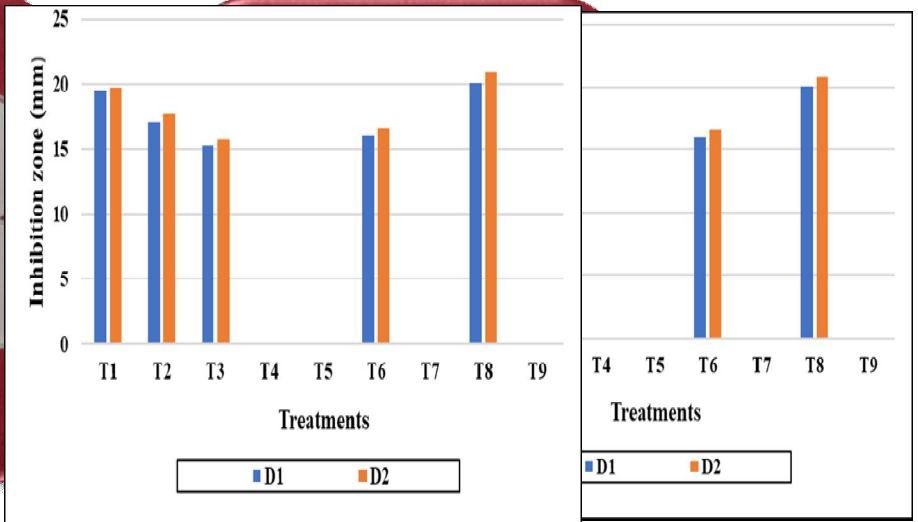
The data assessing the efficacy of various fungicides on potassium solubilizing bacteria, the inhibition zones observed at 48 hours of incubation ranged from 0.00 to 20.11 mm for 50% of the recommended doses and 0.00 to 20.88 mm for the full recommended doses, averaging 0.00 to 20.49 mm. By 72 hours, 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.

**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	
<b>T1</b>	<b>Mancozeb 75% WP</b>	19.50	19.76	19.63	20.11	20.87	20.49
<b>T2</b>	<b>Zineb 75% WP</b>	17.13	17.68	17.40	18.09	18.94	18.51
<b>T3</b>	<b>Metalaxyl 35% WS</b>	15.26	15.81	15.53	16.27	16.89	16.58
<b>T4</b>	<b>Carbendazim 50% WP</b>	00.00	00.00	00.00	00.00	00.00	00.00
<b>T5</b>	<b>Propiconazole 25% EC</b>	00.00	00.00	00.00	00.00	00.00	00.00
<b>T6</b>	<b>Azoxystrobin 18.2% + Difenconazole 11.4% SC</b>	16.05	16.67	16.36	17.13	17.76	17.44
<b>T7</b>	<b>Carbendazim 12 % + Mancozeb 63% WP</b>	00.00	00.00	00.00	00.00	00.00	00.00
<b>T8</b>	<b>Azoxystrobin 11% + Tebuconazole 18.3% SC</b>	20.11	20.88	20.49	21.37	21.93	21.65
<b>T9</b>	<b>Control</b>	00.00	00.00	00.00	00.00	00.00	00.00
	<b>S.E.±</b>	<b>0.41</b>	<b>0.39</b>	-	<b>0.41</b>	<b>0.38</b>	-
	<b>C.D. (P=0.01)</b>	<b>1.70</b>	<b>1.60</b>	-	<b>1.68</b>	<b>1.57</b>	-

\*Mean of three replications

RD = Recommended dose



**50 % of Recommended dose**

**Recommended dose**

Recommended dose

50 % of recommended dose

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

The results obtained in present study are similar with the results of earlier workers, Surendran *et al.* (2012) reported that, compatibility of *Pseudomonas fluorescens* with 15 fungicides 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% + Mancozeb 50% WS were found highly compatible and rest of fungicides were non compatible with *Pseudomonas fluorescens*.

### 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% WP, Metalaxyl 35 % WS, Azoxystrobin 18.2 % + Difenconazole 11.4 % SC, Azoxystrobin 11% + Tebuconazole 18.3 % were incompatible bacterium with the test bacterium. Similar work was done by Ashwini *et al.* (2019) 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)

Option 1:

Author(s) 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.

### References

Ashwini M. B. (2019). *Studies on compatibility of phosphate and potassium solubilizing bacteria with agrochemicals (Master's thesis)* Vasantrao Naik Marathwada Krishi Vidhyapeeth, Parbhani.

Chandra, P. B., Ingle, R. W. & Tetali, S. (2016). Compatibility of Phosphate Solubilizing Microorganisms with different agrochemicals. *Journal of Plant Archives*. 16(1), 229 -232.

Goswami, S. P. & Maurya, B. R. (2019). Impact of potassium solubilizing bacteria (KSB) and sources of potassium on yield attributes of maize (*Zea mays L.*). *Journal of Pharmacognosy and Phytochemistry*. 9(1), 1610-1613.

Hanuman, L. N. & Madhavi, G. B. (2018). Compatibility of *Pseudomonas fluorescens* with pesticides *in vitro*. *International Journal of Current Microbiology and Applied Sciences*. 7(3), 3310- 3315.

Harsha M. K., Daunde, A. T., Bhalerao, P. B. & Sakhare, S. S. (2023). Compatibility studies of *Bacillus spp.* with commonly used agrochemicals. *The PharmaInnovation Journal*. 12(1), 110-114.

Khan, A. A., Jilani, G., Akhtar, M. S., Naqvi, S. M. S. & Rasheed, M. (2009). Phosphorus solubilizing bacteria: occurrence, mechanisms and their role in crop production. *Journal of agricultural biology science*. 1(1), 48-58.

Kurhade, K. C., Gade, R. M., Belkar, Y. K. & Chaitanya, B. H. (2016). Detecting tolerance in *Pseudomonas fluorescens* to pesticides. *Agricultural Science Digest-A Research Journal*. 36(3), 247-249.

Nwanyanwu, C. E., Umeh, S. I. & Sapele, A. (2015). Effect of phosphate solubilizing bacteria on growth characteristics of maize, beans and groundnut seedlings in potted soil. *Nigerian Journal of Microbiology*. 29(3), 3159-3166.

Purushothaman, S. M., RehumathNiza, T. J. & Ravi, S. (2014). *In vitro* interaction between fungicides and beneficial plant growth promoting Rhizobacteria *African Journal of Agricultural Research*. 8(45), 1-4.

Raghavendra, M., Singh, Y. V., Gaiind, S., Meena, M. C. & Das, T. K. (2018). Effect of potassium and crop residue levels on potassium solubilizers and crop

yield under maize-wheat rotation. *International Journal of Current Microbiology and Applied Science*. 7(06), 424-435.

Rajkumar, K., Naik, M. K., Chennappa, G. & Amaresh, Y. S. (2018). Compatibility *Bacillus subtilis* (BS 16) with fungicides used in chilli ecosystem for integrated disease management. *International Journal of Chemical. Studies*. 6(3), 3393-3396.

Surendran, M., Kannan, G. S., Nayar, K. A. M. A. L. A. & Leenakumary, S. (2012). Compatibility of *Pseudomonas fluorescens* with agricultural chemicals. *Journal of Biological Control*. 26(2), 190-193.

Vincent J.M. (1947). Distortion of fungal hypha in the presence of certain inhibitors. *Nature. The Pharma Innovation Journal*. 159(4051), 850-850.

Vishakha K. B. (2019). Studies on compatibility of *Pseudomonas fluorescens* with Agrochemicals (Master's thesis). Vasantrya Naik Marathwada Krishi Vidhyapeeth, Parbhani.

Singh, M., Singh R. and Nagar D. (2021) *In vitro* compatibility of *Pseudomonas fluorescens* with different systemic fungicides. *The Pharma Innovation Journal*. 10(3), 874-877.

Sarvani, B., Reddy, R. S. & Prasad, J. S. (2021). Characterization of plant growth promoting rhizobacteria for compatibility with commonly used Agrochemicals. *Jr of Eco. Env. & Cons.* 27 (8), 76-80.

Dinkwar GT, Thakur KD, Bramhankar SB, Pillai TS, Isokar SS, Ravali T, Kharat VM. In vitro compatibility of *B. japonicum* with fungicides. *IJCS*. 2020;8(2):692-4.