RESEARCH ARTICLE

Compatibility Study of Azotobacter Species with commonly used Agrochemicals in India

Apurba Bera*, Damodhara Chari
Sankalp Taru, West Bengal,India, Plant Health Management, NIPHM, Hyderabad, India
Email: apurba.bera@gmail.com

The present investigation was undertaken to study the compatibility of Azotobacter spp. with commonly used agrochemicals in India. Azotobacter isolates were obtained from the Biofertilizer Laboratory and Biocontrol Laboratory of the National Institute of Plant Health Management (NIPHM), Hyderabad, India. Under laboratory conditions, Azotobacter failed to multiply at the field-recommended dose of fungicides such as Mancozeb 75% WP, Hexaconazole 5% EC, and Propiconazole 25% EC, as no colony formation was observed. However, at half the recommended dose, all the tested fungicides were found to be compatible with Azotobacter. The bacterium was able to multiply at both the field-recommended and half-recommended doses when inoculated with herbicides including Glyphosate 41% SL, 2,4-D Sodium salt 80% WP, and Pretilachlor 50% EC. Similarly, insecticides such as Fipronil 80% WG, Thiamethoxam 25% WG, and Quinalphos 25% EC were found to be compatible with Azotobacter at both dosage levels. Under field conditions, Azotobacter showed multiplication even at the field-recommended doses of fungicides, herbicides, and insecticides, indicating a differential response between laboratory and field environments. Agrochemicals that do not adversely affect the growth of microbial inoculants can be further evaluated and potentially used in combination with biofertilizers. The results suggest that Azotobacter is compatible with several commonly used agrochemicals and can be integrated into Integrated Pest Management (IPM) and Integrated Nutrient Management (INM) systems as a bio-input component.

Keywords: Azotobacter, Nitrogen fixation, Agrochemical compatibility, Biofertilizers, In vivo evaluation, Sustainable agriculture *Rhyzopertha dominica*

Introduction

Agricultural lands become deprived of essential nutrients due to continuous cropping without an adequate supply of organic manure. In addition, many of the nutrients present in the soil are in unavailable forms, which plants cannot utilize directly. Hence, the application of soil nutrients in the form of chemical fertilizers becomes necessary to obtain optimum crop yield. Several studies have demonstrated that the interaction between agrochemicals and beneficial soil microorganisms is highly dependent on the type and concentration of the chemical applied. As a result of the incorporation of agrochemicals, grain production has doubled over the past four decades; however, this has led to unsustainable agricultural practices (Prasad et al., 2024; Kumar & Singh, 2025). Most agrochemicals are not fully absorbed by plants, and a significant portion remains in the environment (Rani et al., 2023). Previous research has shown that biofertilizers and plant growth promoting microorganisms may remain compatible with certain agrochemicals when applied at recommended doses, while higher concentrations often exert inhibitory effects (Meena et al., 2024; Zhang et al., 2026).

Azotobacter is a group of Gram-negative, free-living, nitrogen-fixing aerobic bacteria inhabiting soil. They are oval or spherical in shape and form thick-walled cysts (dormant cells resistant to deleterious conditions) under unfavorable environmental conditions (Bhattacharya et al., 2023). Around six species of the genus Azotobacter have been reported, some of which are motile by means of peritrichous flagella, while others are immotile. They are typically polymorphic, with sizes ranging from 2 to 10 µm in length and 1 to 2 µm in width. The genus Azotobacter was first recognized in 1901 by the Dutch microbiologist and botanist Beijerinck and his co-workers as the first aerobic free-living nitrogen fixer.

These bacteria utilize atmospheric nitrogen for cellular protein synthesis, which is subsequently mineralized in the soil, thereby supplying a considerable amount of nitrogen to crop plants. Azotobacter is sensitive to acidic pH, high salt concentrations, and extreme temperatures (Sarkar & Das, 2024). These organisms exert beneficial effects on crop growth and yield through the biosynthesis of biologically active substances, stimulation of rhizospheric microbes, production of phytopathogenic inhibitors, alteration of nutrient uptake, and enhancement of biological nitrogen fixation (Lenart, 2012; Zhao et al., 2025; Gupta et al., 2023).

Materials and Methods

Materials

Microbial agents

Microbial bio-inoculants, viz., Azotobacter, were collected and purified from the Plant Pathology and Biocontrol Laboratories of NIPHM and were used for this study.

Agrochemicals

Different agrochemicals, including fungicides such as Mancozeb 75% WP, Hexaconazole 5% EC, and Propiconazole 25% EC; herbicides such as Glyphosate 41% SL, 2,4-D Sodium salt 80% WP, and Pretilachlor 50% EC; and insecticides such as Fipronil 80% WG, Thiamethoxam 25% WG, and Quinalphos 25% EC, were collected from the Pesticide Management Division, NIPHM, and used for this study.

Pot mixture materials and pots

Red soil, sand, farmyard manure, vermicompost, and pots (plastic cups) required for preparing the pot mixture for the experiment were arranged by the institute.

Methods

The experiment was conducted in two sets, and the details are furnished below.

Published online: 01 February 2026

In vitro experiment

Collection of beneficial microbial cultures

Azotobacter microbial cultures (biofertilizers) were collected and purified from the laboratories of NIPHM.

Collection of different agrochemicals

Commonly used agrochemicals such as insecticides, fungicides, and herbicides were collected from the Pesticide Management Division, NIPHM.

Compatibility study

The compatibility of different cultures with commonly used agrochemicals was assessed by measuring the colony count of bacterial colonies on selected media plates using the poisoned food technique (Nene, 1971).

Fungicides, insecticides, and herbicides at different concentrations were used for the in vitro assay. The fungicides, insecticides, and herbicides were incorporated into the sterile medium. Sterilized Petri plates containing the amended medium were inoculated with bacterial culture (0.1 ml or 1 ml) of freshly prepared test culture and incubated at 28 ± 1°C for 7 days. The efficacy of agrochemicals was expressed as the percentage of colony count over the control.

In vivo experiment

Collection of beneficial microbial cultures

Different types of beneficial microbial cultures (biofertilizers and biopesticides) were collected and purified from the laboratories of NIPHM.

Collection of different agrochemicals

Commonly used agrochemicals such as insecticides, fungicides, and herbicides were collected from the Pesticide Management Division, NIPHM. Field soil was collected and sterilized using an autoclave for two successive days. After sterilization, the soil was mixed with agrochemicals and bio-inoculant cultures. Soil samples were collected at weekly intervals, and microbial viable counts were performed for the different treatments.

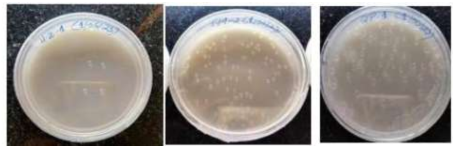

Figure 1: Growth of Azotobacter under in vitro conditions with agrochemicals

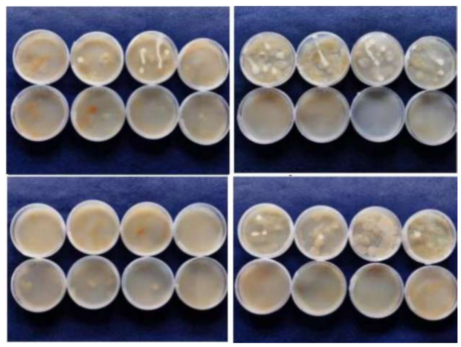

Figure 2: Growth of Azotobacter under in vivo conditions with agrochemicals

Table 1: Agrochemicals and Their Concentrations Used for Compatibility Studies

Agrochemical Name	Field RD	Half RD	Test A	Test B
	Fungicides			
Mancozeb 75% WP	2.5 g/L	1.25 g/L	0.25 g	0.125 g
Hexaconazole 5% EC	2 ml/L	1 ml/L	0.2 ml	0.1 ml
Propiconazole 25% EC	2 ml/L	1 ml/L	0.2 ml	0.1 ml
	Herbicides			
Glyphosate 41% SL	6 ml/L	3 ml/L	0.6 ml	0.3 ml
2,4-D Sodium salt 80% WP	5 g/L	2.5 g/L	0.5 g	0.25 g
Pretilachlor 50% EC	2 ml/L	1 ml/L	0.2 ml	0.1 ml
	Insecticides			
Fipronil 80% WG	0.3 g/L	0.15 g/L	0.03 g	0.015 g
Thiamethoxam 25% WG	2 g/L	1 g/L	0.2 g	0.1 g
Quinalphos 25% EC	2.5 ml/L	1.25 ml/L	0.25 ml	0.125 ml

Results

Effect of Different Agrochemicals under In Vitro Experiments on Microbial Bio-inoculant viz., Azotobacter spp.

Test: Azotobacter + 9 Agrochemicals

At the field-recommended dose, Azotobacter was not able to be multiplied when inoculated with fungicides such as Mancozeb 75% WP, Hexaconazole 5% EC, and Propiconazole 25% EC, and with the insecticide Quinalphos 25% EC, as no colonies were observed. However, at the half-recommended dose, all four pesticides were found to be compatible with Azotobacter.

It was also observed that Azotobacter was able to multiply when inoculated with herbicides such as Glyphosate 41% SL, 2,4-D Sodium salt 80% WP, and Pretilachlor 50% EC, as well as insecticides such as Fipronil 80% WG and Thiamethoxam 25% WG, at both the field-recommended dose and half-recommended dose, indicating compatibility with Azotobacter under in vitro conditions.

Effect of Different Agrochemicals under In Vivo Experiments on Microbial Bio-inoculant viz., Azotobacter spp.

Test: Azotobacter + 9 Agrochemicals

Under in vivo conditions, at both the field-recommended dose and half-recommended dose, Azotobacter was able to multiply when inoculated with fungicides such as Mancozeb 75% WP, Hexaconazole 5% EC, and Propiconazole 25% EC; herbicides such as Glyphosate 41% SL, 2,4-D Sodium salt 80% WP, and Pretilachlor 50% EC; and insecticides such as Fipronil 80% WG, Thiamethoxam 25% WG, and Quinalphos 25% EC. In sterilized soil, the growth of Azotobacter colonies was lower compared to non-sterilized soil.

Published online: 01 February 2026

Conclusions

The present study evaluated the compatibility of the bio-inoculant Azotobacter spp. with commonly used agrochemicals under both *in vitro* and *in vivo* conditions. The results clearly indicate that the response of Azotobacter to agrochemicals is dose-dependent and varies according to the class of chemical applied.

Under laboratory conditions, Azotobacter exhibited reduced or inhibited growth at the field-recommended doses of certain fungicides; however, compatibility was consistently observed at half-recommended doses. In contrast, herbicides such as Glyphosate 41% SL, 2,4-D Sodium salt 80% WP, and Pretilachlor 50% EC, as well as insecticides including Fipronil 80% WG and Thiamethoxam 25% WG, supported the multiplication of Azotobacter at both the field-recommended and half-recommended doses, indicating a high degree of compatibility.

Under *in vivo* conditions, Azotobacter was able to multiply in soil even at the field-recommended doses of fungicides, herbicides, and insecticides, demonstrating a differential response between controlled laboratory conditions and soil ecosystems. This observation suggests that soil-related factors may alleviate the adverse effects of certain agrochemicals on microbial bio-inoculants.

Overall, the study confirms that agrochemical compatible Azotobacter strains can be effectively integrated with selected agrochemicals without adversely affecting microbial viability. These findings are valuable for the development of bio-inoculant formulations and support the practical application of Azotobacter in Integrated Pest Management (IPM) and Integrated Nutrient Management (INM) strategies, contributing to more sustainable agricultural production systems.

Received: 13 January 2026 **Revised:** **Accepted:** 16 January 2026 **Published:** 31 January 2026

Table 2: In vitro compatibility of *Azotobacter* with insecticides, fungicides, and herbicides

Group	Agrochemical	Field RD	Half RD
Insecticide	Fipronil 80% WG	M1, M2, M3–TMC	M1, M2, M3–TMC
	Thiamethoxam 25% WG	M1–192, M2–104, M3–140	M1, M2, M3–TMC
	Quinalphos 25% EC	M1, M2, M3–NIL	M1–152, M2–132, M3–204
Fungicide	Mancozeb 75% WP	NIL	M1, M2, M3–TMC
	Hexaconazole 5% EC	NIL	M1–4, M2–2, M3–1
	Propiconazole 25% EC	NIL	M1, M2, M3–TMC
Herbicide	Glyphosate 41% SL	M1–8, M2–11, M3–7	M1–156, M2–140, M3–184
	2,4-D Sodium salt 80%WP	M1–128, M2–172, M3–368	M1, M2, M3–TMC
	Pretilachlor 50% EC	M1–3, M2–4, M3–5	M1, M2, M3–TMC

Table 3: In vivo compatibility of *Azotobacter* with insecticides, fungicides, and herbicides

Group	Agrochemical	Field RD	Half RD
Insecticide	Fipronil 80% WG	M1, M2, M3–TMC	M1, M2, M3–TMC
	Thiamethoxam 25% WG	M1, M2, M3–TMC	M1, M2, M3–TMC
	Quinalphos 25% EC	M1, M2, M3–TMC	M1, M2, M3–TMC
Fungicide	Mancozeb 75% WP	M1, M2, M3–TMC	M1, M2, M3–TMC
	Hexaconazole 5% EC	M1, M2, M3–TMC	M1, M2, M3–TMC
	Propiconazole 25% EC	M1, M2, M3–TMC	M1, M2, M3–TMC
Herbicide	Glyphosate 41% SL	M1, M2, M3–TMC	M1, M2, M3–TMC
	2,4-D Sodium salt 80% WP	M1, M2, M3–TMC	M1, M2, M3–TMC
	Pretilachlor 50% EC	M1, M2, M3–TMC	M1, M2, M3–TMC

References

Nene, Y. L. (1971). *Fungicides in Plant Disease Control*. Oxford & IBH, New Delhi, India.

Sumbul, A., Ansari, R. A., Rizvi, R., & Mahmood, I. (2020). Azotobacter: A potential bio-fertilizer for soil and plant health management. *Saudi Journal of Biological Sciences*, 27(12), 3634–3640. https://doi.org/10.1016/j.sjbs.2020.08.004

Moses Martin, & Rajesh Kannan. (2011). Influence of agrochemicals and Azotobacter application on soil fertility in relation to maize growth under nursery conditions. *Journal of Advanced Biotech*, 10(12), 18-21.

Prasad, R., et al. (2024). Sustainable soil management and the transition from chemical to bio-based fertilizers. *Journal of Cleaner Production*, 410, 137251. https://doi.org/10.1016/j.jclepro.2024.137251

Rani, S., et al. (2023). Environmental persistence of modern pesticides and their leaching kinetics in agricultural soils. *Chemosphere*, 315, 137780. https://doi.org/10.1016/j.chemosphere.2023.137780

Meena, M. K., et al. (2024). Synergistic effects of biofertilizers and low-dose pesticides on maize growth. *Applied Soil Ecology*, 194, 105190. https://doi.org/10.1016/j.apsoil.2023.105190

Sarkar, A., & Das, T. (2024). Resilience of Azotobacter species to salinity and thermal stress in tropical soils. *Microbial Ecology*, 87(2), 45. https://doi.org/10.1007/s00248-023-02315-w

Zhao, Y., et al. (2025). Mechanisms of atmospheric nitrogen fixation in non-symbiotic bacteria: A molecular update. *Nature Communications*, 16, 204. https://doi.org/10.1038/s41467-024-55892-z

Vyas, et al. (2020). Compatibility study of fungal and bacterial bio control agents with agrochemicals. *International Journal of Current Microbiology and Applied Sciences*, 9(4), 1152–1159. https://doi.org/10.20546/ijcmas.2020.904.136

Shahid, S., & Saghir Khan. (2022). Tolerance of pesticides and antibiotics among beneficial soil microbes recovered from contaminated rhizosphere of edible crops. *Current Research in Microbial Sciences*, 3, 100091. https://doi.org/10.1016/j.crmicr.2021.100091

Dhanya, M. K., Anjumol, K., Murugan, M., & Deepthy, K. B. (2017). Compatibility of *Trichoderma viride* and *Pseudomonas fluorescens* with plant protection chemicals and fertilizers in cardamom. *Journal of Tropical Agriculture*, 54, 129–134.

Published online: 01 February 2026