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EFFECTS OF COMMON HERBICIDES ON DIVERSITY OF PLANT GROWTH-PROMOTING RHIZOBACTERIA IN CROP FIELDS

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ABSTRACT Plant Growth-Promoting Rhizobacteria (PGPR) offer a promising solution to significantly increase crop productivity without causing harm to the environment. They improve the availability of nutrients, maintain plant health, and increase soil fertility as an integral part of sustainable agricultural practices. The vigorous use of herbicides in modern agriculture has damaging effects on PGPR populations and their functions. The reduction in PGPR diversity leads to altered metabolic activities and disrupting nutrient cycling that negatively impacts crop health and yield. Therefore, it is crucial to develop sustainable weed management practices to mitigate adverse effects on beneficial soil microflora. The study was conducted in Ranigaon Village of Bilaspur District, Chhattisgarh, India. We have isolated bacterial genera viz., *Pseudomonas, Bacillus, Azospirillum* and *Rhizobium* form a rhizospheric soil sample as PGPR per literature and experimental design. The effect of 2-4 D herbicide in PGPR was recorded with a maximum percentage growth inhibition of 67.49 ±0.70 (60 mg L^{-1}) with Rhizobium sp. while a minimum of 60.13 ±0.57 (60 mg L-1) percentage growth inhibition with *Bacillus* sp. The variance among growth inhibition of *Pseudomonas* sp., *Bacillus* sp., *Azospirillum* sp., and *Rhizobium* sp. was noted at 130.10. The present research outcome urges the adaptation of sustainable agricultural practices to ensure the minutest use of herbicides to protect soil health. However, systematic scientific evidence is required to understand the interaction between herbicides and PGPR to establish an adequate agricultural foundation for farmers.

KEYWORDS : PGPR, Sustainable Agricultural Practices, Nutrient Cycling, Nutrient Uptake, *Bacillus* sp., *Azospirillum* sp., *Pseudomonas* sp., *Rhizobium* sp.

INTRODUCTION

Plant Growth-Promoting Rhizobacteria (PGPR) often colonize plant roots and induce plant growth through various mechanisms, such as nitrogen fixation, phosphate solubilization, and the synthesis of numerous plant growth regulators, i.e., auxins, gibberellins, and cytokinins (Glick, 2012). The PGPR has been associated with improved crop yields, particularly in cereals, legumes, and vegetables. These bacteria play a vital role in sustainable agriculture by enhancing the availability of nutrients, maintaining plant health, and enhancing soil fertility. The application of PGPR in crop fields has gathered significant attention nowadays as an eco-friendly alternative to synthetic fertilizers and pesticides (Vessey, 2003). Nitrogen-fixing bacteria like *Rhizobium* and *Azospirillum* contribute to atmospheric nitrogen fixation; thereby, plants readily absorb and utilize the fixed nitrogen, and hence, this reduces the necessity for synthetic nitrogen fertilizers (Hungria *et al.*, 2010; Bhattacharyya and Jha, 2012) in crop fields. Likewise, phosphate-solubilizing bacteria, such as *Pseudomonas* and *Bacillus* species, release organic acids that effectively solubilize bound phosphates in the soil and make them available to plants (Rodríguez et al., 2006; Bhattacharyya and Jha, 2012). This process fixes phosphate and significantly increases its availability to crops. Moreover, PGPR produces siderophores that facilitate the chelation of iron in the soil, which makes iron more available to plants and also helps plants to outcompete harmful pathogens. Additionally, some PGPR strains have been reported to produce antibiotics and a variety of lytic enzymes that suppress plant pathogens and reduce the incidence of soil-borne diseases (Compant et al., 2005).

Research has shown that the inoculation of PGPR in crop fields improves soil structure and fertility, enhancing microbial diversity, which further induces nutrient cycling and overall soil health (Bhattacharyya & Jha, 2012). For example, studies have reported yield increases of up to 30% in wheat and maize when inoculated with PGPR (Maheshwari, 2011). PGPR bacteria also help protect plants against pathogens (Vessey, 2003). Adopting organic farming practices and reduced chemical inputs can further promote the diversity of PGPR, enhancing their beneficial effects on crop health and yield (Lopes et al., 2021). Continued research into the diversity and function of PGPR in various agroecosystems is essential to optimizing their use in sustainable crop production.

Herbicides viz., Glyphosate (Duke & Powles, 2008), Atrazine (Solomon et al., 1996), Paraquat (Duke & Powles, 2008), 2,4-Dichlorophenoxyacetic acid (Grossmann, 2010), and Pendimethalin (LeBaron & McFarland, 1990) have widely been reported to control weeds in crop fields. Herbicides have been divulged for harmful action against PGPR, e.g., glyphosate has been noted to inhibit the growth of PGPR viz., *Pseudomonas* and *Bacillus* species that are involved in nitrogen fixation and phosphate solubilization respectively (Kremer & Means, 2009). Likewise, the herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) has been reported to alter the balance of soil microbiota, including PGPR communities (Zabaloy et al., 2010). Mijangos et al. (2009) revealed that the herbicide-exposed PGPR has reduced IAA synthesis rate, which leads to underdeveloped roots and, thereby, poor nutrient uptake by plants. Herbicides also interfere with the secretion of siderophores by PGPR (Zhang et al., 2013). Hence, the uncontrolled use of herbicides can disrupt nutrient cycling in the soil by affecting PGPR activities.

MATERIALS AND METHODS

The investigation was conducted in Ranigaon Village (Geographical coordination: 22.2589383374, 82.1436196788), under Ratanpur Block of Bilaspur District, Chhattisgarh, India. A rhizospheric soil sample was collected to isolate PGPR bacteria. In the present study, 2,4-D was used as an herbicide to examine its effect on isolated PGPR.

Isolation And Screening Of PGPR bacteria

As per the literature, *Bacillus* sp., *Azospirillum* sp., *Pseudomonas* sp., and *Rhizobium* sp. were isolated from the soil samples and considered PGPR bacteria (Hungria et al., 2010; Rodríguez et al., 2006; Bhattacharyya and Jha, 2012). The soil samples were subjected to serial dilution and 0.5 ml of 10^{-5} serially diluted samples were inoculated onto different media viz., *Azospirillum* Medium (with 0.17% Agar and malate), *Rhizobium* Medium (0.1 % mannitol), and Nutrient agar media (for *Bacillus* sp. and *Pseudomonas* sp.) and incubated at 37°C) for 48 h. After the incubation period, the pure culture of each bacterial strain was prepared and further proceeded for screening and identification. The PGPR bacteria were screened and identified at the Genus level using the Key mentioned in Bergey's Manual of Determinative Bacteriology (Holt et al., 1994) regarding Colony, Microscopic and Biochemical features.

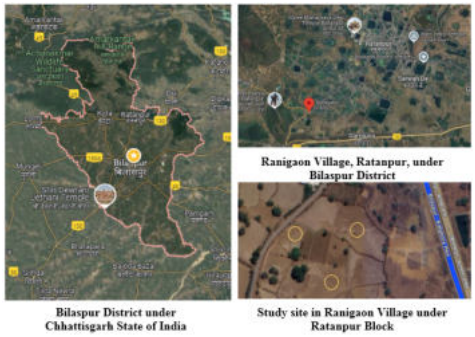

Fig 1. A geographical Representation of the study site

EFFECT OF HERBICIDES IN PGPR

The PGPR isolates *Bacillus* sp., *Azospirillum* sp., *Pseudomonas* sp., and *Rhizobium* sp. were further evaluated for the effect of herbicide 2,4-D at a range of 20-50 mg L^{-1}. The bacterial culture at the log phase was inoculated with herbicide as per the experimental design, and their growth curve was spectrophotometrically (at 600 nm) examined.

The collected data were tabulated and analyzed using MS Office 2021. The mean value and standard deviation for all the data sets were prepared using the MS Excel formula.

RESULTS AND DISCUSSION

The research was conducted in Ranigaon Village of Bilaspur District, Chhattisgarh, India. A rhizospheric soil sample was collected to isolate PGPR bacteria. The bacterial genera viz., *Pseudomonas*, *Bacillus*, *Azospirillum* and *Rhizobium* were isolated from the samples as the PGPR bacterial group as per the key mentioned by Bergey's Manual of Determinative Bacteriology. The Colony, Microscopic and Biochemical Characterization of PGPR isolates are mentioned in Table 1. Further, the 2,4-D was used as an herbicide to examine its effect on isolated PGPR. The effect of 2-4 D herbicide in PGPR is shown in Table 2. *Rhizobium* sp. has been noted with maximum percentage growth inhibition of 67.49 ±0.70, whereas *Bacillus* sp. was observed with minimum percentage growth inhibition of 60.13 ±0.57, with 60 mg L-1 of 2,4-D correspondingly (Fig 2).

Table 1. Colony, Microscopic and Biochemical Characterization of PGPR isolates

Colony, Microscopic and Biochemical Characterization	***Bacillus* sp.**	***Pseudomonas* sp.**	***Azotobacter* sp.**	***Rhizobium* sp.**
Colony Color	Cream	Off White	Cream	Off White
Colony shape	Jagged Edges	Irregular Margins	Irregular Margins	Round
Grams reaction	+	-	-	-
Shape	Rod	Rod	Rod	Rod
Catalase	+	+	+	+
Citrate utilization	+	+	+	-
Gelatin Hydrolysis	+	+	-	-
H2S production	-	-	+	+
Indole	-	-	+	+
Mannitol	+	-	+	+
Methyl red	-	-	+	+
Nitrate reduction	+	+	+	+
Oxidase	-	+	+	+
Starch Hydrolysis	+	+	+	+
Vogues Proskauer	+	-	+	+

The statistical analysis of percentage growth inhibition results revealed that the variance among growth inhibition of PGPR isolates was 130.10. The correlation of growth inhibition between *Rhizobium* sp. to *Pseudomonas* sp., *Rhizobium* sp. to *Bacillus* sp., and *Rhizobium* sp. to *Azospirillum* sp. were observed at 0.954, 0.950, and 0.917 respectively. The fold increase in growth inhibition in the presence of herbicide at a predefined range in *Pseudomonas* sp., *Bacillus* sp. *Azospirillum* sp. and *Rhizobium* sp. were recorded at 2.01, 2.02, 1.69, and 1.59, respectively. The details of statistical analysis for percentage growth inhibition of PGPR with 2,4-D at different concentration ranges from 20 mg L^{-1} to 50 mg L^{-1} at intervals of 10 are shown in Table 2.

Herbicides are extensively utilized in agricultural practices to control weeds in crop fields, but their negative effect on beneficial soil microflora have been scientifically proven and documented. Literature divulged that Glyphosate (10 mg L^{-1}) inhibit P. fluorescens by 30% (Zabaloy et al., 2012), Atrazine (15 mg L^{-1}) to Bacillus subtilis by 45% (Omar & Abdel-Sater, 2001), Paraquat (20 mg L^{-1}) to A. brasilense by 60% (Santos et al., 2006), 2,4-D (25 mgL^{-1}) to R. leguminosarum by 50% Riaz et al., 2013; Pendimethalin (30 mgL^{-1}) to Enterobacter cloacae by 40% (Ahemad & Khan, 2011). The bacterial strains often exhibit the highest growth rate in the log phase.

Table 2. Statistical analysis for percentage growth inhibition of PGPR with 2,4-D at different concentration ranges from 20 mg L^{-1} to 50 mg L^{-1} at interval of 10.

S. No.	Statistical analysis for growth inhibition of PGPR with 2,4-D	Value
1.	Variance among growth inhibition of *Pseudomonas* sp., *Bacillus* sp., *Azospirillum* sp., *Rhizobium* sp.	130.10
2.	Correlation of growth inhibition between *Rhizobium* sp. to *Pseudomonas* sp.	0.954
3.	Correlation of growth inhibition between *Rhizobium* sp. to *Bacillus* sp.	0.950
4.	Correlation of growth inhibition between *Rhizobium* sp. to *Azospirillum* sp.	0.917
5.	Fold increase in growth inhibition in the presence of herbicide at a predefined range in *Pseudomonas* sp.	2.01
6.	Fold increase in growth inhibition in the presence of herbicide at a predefined range in *Bacillus* sp.	2.02
7.	Fold increase in growth inhibition in the presence of herbicide at a defined range in *Azospirillum* sp.	1.69
8.	Fold increase in growth inhibition in the presence of herbicide at a predefined range in *Rhizobium* sp.	1.59

Studies have shown that herbicides have direct and indirect effects on PGPR. The direct effects include inhibiting PGPR growth and activity by interrupting their metabolic operation or creating toxic conditions in the soil; in contrast, the indirect effects comprise alteration of the soil environment (e.g., soil pH, nutrient availability, microbial diversity) that induces unfavourable conditions for PGPR growth and survival (Beneduzi et al., 2012).

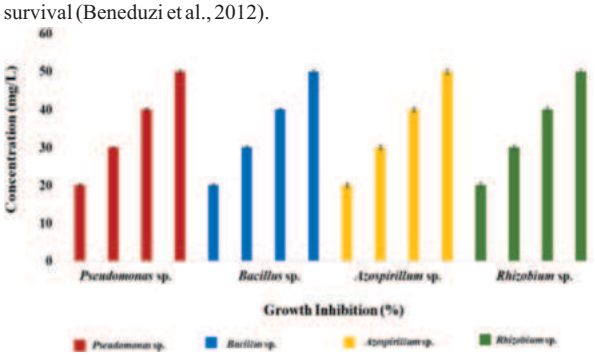

Fig 2. Effect of 2-4 D herbicide in PGPR isolates

Herbicides have exhibited inhibitory action against the nitrogen-fixing ability of PGPR, i.e., *Azospirillum* species (Rosenbaum et al., 2017) and also alter the ability of PGPR to solubilize phosphorus (Singh & Walker, 2006). These scenarios lead to nutrient deficiencies in crop fields. However, PGPR species, e.g., *Pseudomonas*, have been observed to adapt against the herbicide, viz., atrazine stress, by developing resistance (Radosevich *et al.*, 1995). Nevertheless, concerns arise towards developing potent herbicide-resistant weeds by moving genetic information between bacteria.

CONCLUSION

Herbicides are an essential part of weed control in India nowadays. However, it is crucial to consider their potential impacts on PGPR. Reducing these beneficial bacteria can negatively impact soil health and plant growth, as these PGPRs are essential for maintaining nutrient cycles and promoting plant vigour. The present situation urges sustainable agricultural practices to ensure minimum use of herbicides to protect soil health. These practices warrant long-term sustainability in agriculture. The interaction between herbicides and PGPR is complicated and demands more scientific evidence to develop effective management practices.

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