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Analyzing the Rhizosphere Microbiome in Pesticide-Contaminated Soil for Microbial Bioremediation

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Abstract **- The rise in agricultural activity has led to widespread herbicide use, including glyphosate, which poses risks to soil health and the symbiosis between plants and microbes. This study investigated the effects of glyphosate at a concentration of 100 ppm on the rhizosphere of chickpea plants (*Cicer arietinum L.*) using soil samples collected from Nagpur, Maharashtra. Results indicated a significant reduction in both total DNA and protein levels in treated groups (P1, P2, P3) compared to control groups (NP1, NP2, NP3). Furthermore, there was a noticeable decline in the number of root nodules in treated chickpeas, suggesting a disruption in the symbiotic relationship between the plants and microbes. Metagenomic analysis was performed utilizing the Illumina MiSeq sequencing platform (BioProject: PRJNA1258555), demonstrating the rhizosphere of legume plants is sensitive to elevated pesticide levels.**

Keywords **- Glyphosate, chickpea rhizosphere, soil metagenomics, root nodules, herbicide effects.**

I. INTRODUCTION

Pesticide contamination presents a significant environmental challenge that must be addressed to promote sustainable agriculture and preserve soil biodiversity [1]. Glyphosate [N-(phosphonomethyl) glycine] is the most widely utilized herbicide due to its effectiveness in weed control [2]. However, its persistence in soil and its capacity to bind essential minerals can adversely affect the chemical and biological properties of the rhizosphere, which are the nutrient-rich area surrounding plant roots and a key site for microbial activity [3]. Chickpeas (*Cicer arietinum*) are particularly sensitive to soil and microbial health, largely due to their symbiotic relationship with rhizobia for nitrogen fixation [4]. Prior research has shown that glyphosate can disrupt physiological and molecular mechanisms in plants and their microbial associations, reducing beneficial bacteria and mycorrhizal fungi, potentially resulting in yield losses and increased susceptibility to diseases [5,6]. Nonetheless, there is limited research examining the impact of high levels of glyphosate on the chickpea rhizosphere microbiome in various regions, including Nagpur, India. This study aimed to explore the microbial community in pesticide-affected soil and assess the feasibility of bioremediation.

II. MATERIALS AND METHODS

2.1 Soil Collection and Experimental Setup

Soil samples were collected from agricultural fields in Nagpur, Maharashtra, India, and characterized before conducting experiments. A pot experiment with chickpea seeds was set up in two groups: the treated group (P1, P2, P3) received pesticide treatment, while the control group (NP1, NP2, NP3) did not.

2.2 Pesticide Treatment

The treated group was administered a glyphosate dose of 100 ppm, deemed significant due to its potential occurrence in agricultural mishaps.

2.3 Sample Processing and Biochemical Evaluation

Rhizosphere soil samples were extracted from the roots of chickpea plants post-treatment. Total DNA was isolated using standard soil DNA extraction kits, and protein content was assessed to gauge microbial biomass and activity. The root systems were observed for nodule presence.

2.4 Metagenomic Sequencing

To analyze microbial community structure, DNA extracted from samples was sequenced using the Illumina MiSeq platform, focusing on the V3-V4 region of the 16S rRNA gene, a recognized marker for bacterial and archaeal identification. Sequencing data were submitted to the NCBI BioProject database under accession number PRJNA1258555.

III. RESULTS

3.1 Biochemical Effects of Glyphosate

The application of 100 ppm glyphosate significantly affected the biological content of rhizosphere soil. Treated samples (P1, P2, P3) exhibited decreases in total DNA concentration relative to control (NP1, NP2, NP3), correlating with reduced protein levels, indicating diminished biological activity.

3.2 Effects on Chickpea Symbiosis

Morphological observations showed that glyphosate treatment reduced root nodule formation.

Healthy nodules in control plants were pink due to nitrogen fixation, whereas treated plants displayed necrotic nodules, suggesting glyphosate may impede the Rhizobium-chickpea symbiotic interaction.

3.3 Microbial Community Structure

Metagenomic analysis revealed significant alterations in the microbial community within the rhizosphere. Despite confirming the presence of major phyla such as *Proteobacteria*, *Actinobacteria*, and *Firmicutes*, the glyphosate-treated samples showed notable differences in microbial diversity compared to controls, reflecting trends seen in pesticide-affected agroecosystems. Interestingly, key genera associated with bioremediation, such as *Pseudomonas* and *Bacillus*, remained prevalent in contaminated samples, and although their abundance declined due to reduced overall biomass.

IV. DISCUSSION

4.1 Reduction in Microbial Biomass and Symbiotic Relationships

The observed declines in DNA and protein content align with existing literature illustrating how herbicides can suppress rhizosphere microbial communities. Glyphosate's reported ability to diminish viable bacteria and actinomycetes populations underscores its detrimental impact. The decay of nodules indicates that high glyphosate concentrations disrupt signaling pathways critical for chickpea's interaction with nitrogen-fixing bacteria. While some microbial populations may exhibit resistance to herbicide effects, the 100 ppm concentration in the Nagpur soil far exceeds levels conducive to maintaining symbiotic activities. [8][9][12][10][11]

4.2 Microbial Responses and Biodegradation Mechanisms

The shifts in microbial populations suggest glyphosate exerted selective pressure. Certain microbes capable of utilizing glyphosate as a carbon or phosphorus source may have a competitive edge in contaminated environments. For instance, *Pseudomonas alcaligenes* Z1-1 has demonstrated efficiency in degrading glyphosate via specific metabolic pathways. Similarly, fungal species like *Aspergillus tubingensis* have been identified for glyphosate biodegradation, indicating the intrinsic capacity for bioremediation, albeit in a suppressed form.

4.3 Bioremediation Opportunities

Understanding the rhizosphere microbiome provides insights into potential bioremediation strategies. Approaches like biostimulation or the deployment of engineered microbial consortia could enhance glyphosate degradation.

Advances in omics, including metagenomic approaches from BioProject PRJNA1258555, can help identify core degrading genes and pathways vital for soil health recovery. Utilizing Plant Growth Promoting Rhizobacteria (PGPR) that tolerates glyphosate may mitigate its adverse effects on chickpea growth while facilitating pesticide breakdown.

V. CONCLUSION

The findings highlight the negative impact of high glyphosate concentrations on the chickpea rhizosphere, resulting in diminished microbial biomass and impaired nodulation. The experiment in Nagpur has demonstrated the vulnerability of legume symbiosis to pesticide stress, yet it has also revealed the potential to harness resilient degrading microbes for soil bioremediation. Future research should concentrate on cultivating microbial consortia capable of thriving in contaminated soils to restore balance for sustainable chickpea production. [9][11][12][10][7][13]

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