

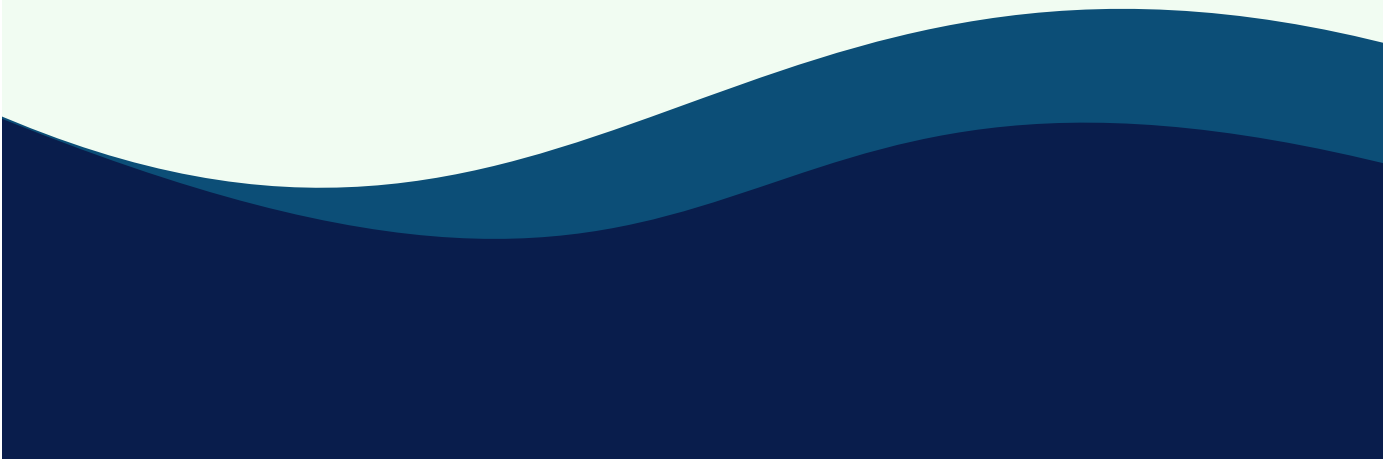
Effect of Herbicides on Agriculturally Beneficial Microorganisms and Their Ecological Functions in Rice-Wheat Cropping System

Himanshu Mahawar
K K Barman





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Cover Photograph

The cover depicts herbicide application in a wheat crop, representing chemical weed management in the rice-wheat system. Hexagonal panels display key microbial indicators: (bottom to up) fungal and *Pseudomonas* colonies, fungal conidiophores, and zones of inhibition by phosphate-solubilizing and cellulose-degrading bacteria, respectively, highlighting the impact of herbicides on beneficial soil microorganisms.

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Foreword

Effective weed management is fundamental to enhancing crop productivity, optimizing input use, and ensuring national food security. The rice-wheat cropping system (RWCS) is the predominant agricultural system in the country. In this system, herbicides are increasingly used due to their broad-spectrum weed control, ease of application, and cost-effectiveness. However, their long-term ecological implications are becoming a growing concern.

One key area of concern is the impact of herbicides on agriculturally beneficial soil microorganisms, including plant growth-promoting rhizobacteria (PGPR), arbuscular mycorrhizal (AM) fungi, phosphate-solubilizing microbes, and biocontrol agents such as *Trichoderma* spp. These microorganisms play critical roles in essential soil ecological functions, such as nutrient mineralization, organic matter decomposition, disease suppression, and the maintenance of soil structure and fertility. Disruptions in microbial community composition and function due to herbicide exposure may compromise soil resilience, ultimately affecting the sustainability of the agroecosystem.

This technical bulletin, *Effect of Herbicides on Agriculturally Beneficial Microorganisms and Their Ecological Functions in the Rice-Wheat Cropping System*, presents a comprehensive synthesis of current scientific research conducted at the ICAR-Directorate of Weed Research, Jabalpur. It aims to elucidate the interactions between chemical weed control practices and soil microbial dynamics within the RWCS, providing evidence-based insights to support integrated and ecologically sustainable weed management.

We commend the authors and the research team for their rigorous efforts in advancing our understanding of the ecological dimensions of herbicide use. This bulletin is expected to serve as a valuable reference for researchers, extension scientists, and policymakers committed to harmonizing agricultural productivity with environmental stewardship.


(A.K. Nayak)



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Preface

Sustainable agriculture faces the dual challenge of increasing food production to meet the needs of a growing global population, projected to reach 10 billion by 2050, while preserving environmental integrity. As per FAO estimates, global food production must rise by approximately 70% to meet future demand. This has driven the intensification of agriculture, especially in India, where high-input systems are widely adopted to enhance productivity. In this context, chemical weed management has emerged as a key strategy, particularly in the intensively cultivated rice-wheat cropping system (RWCS). Herbicides are favored for their efficacy, economic viability, and ease of application, and now represent about 16% of the global pesticide market and 15% of pesticide use in India, dominated by applications in rice (31%) and wheat (44%). Since the introduction of synthetic herbicides like 2, 4-D in the 1940s, these compounds have significantly transformed weed management practices. However, their increasing use raises concerns regarding unintended ecological consequences, particularly the impact on agriculturally beneficial soil microorganisms involved in nutrient cycling, soil health maintenance, and disease suppression.

The RWCS, covering over 10.5 million hectares in India, is vital for national food security. Yet, its sustainability is threatened by the non-judicious use of agrochemicals, including herbicides, which may disrupt soil microbial communities and compromise ecosystem functions. This technical bulletin, *“Effect of Herbicides on Agriculturally Beneficial Microorganisms and Their Ecological Functions in Rice-Wheat Cropping System”*, synthesizes current scientific evidence on herbicide-microbe interactions. It examines how different herbicides influence microbial diversity, abundance, and functional attributes, ranging from suppressive to neutral or even stimulatory effects. Understanding these dynamics is essential for designing ecologically balanced and sustainable weed management practices.

This bulletin serves as a valuable resource for researchers, academicians, extension professionals, farmers, and policymakers, aiming to facilitate informed decision-making within the framework of the Rice-Wheat Cropping System (RWCS). We gratefully acknowledge the significant scientific contributions of Dr. P. J. Khankhane, Dr. V. P. Singh, Dr. Chandra Bhan, Dr. Anil Dixit, Dr. C. Sarathambal, Dr. Shobha Sondhia, Dr. V. K. Choudhary, Dr. Jay G. Varshney and Dr. J. S. Mishra, in advancing research on tillage practices, weed management, and soil microbial dynamics under RWCS. We also extend our sincere appreciation to the project staff, research associates, senior research fellows, and young professionals for their unwavering commitment and dedicated efforts throughout the course of the study.

(Authors)



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01

Introduction

The Rice-Wheat Cropping System (RWCS) is among the most extensive and influential agricultural production systems globally, spanning approximately 24 million hectares across South and East Asia, including India, China, Bangladesh, Nepal, and Pakistan. It plays a central role in ensuring food security and sustaining rural livelihoods in these regions. India is a key contributor to this system, accounting for 9.2 million hectares under RWCS cultivation. During 2023–2024, rice and wheat were cultivated on 47 and 31 million hectares, respectively, in the country, yielding 137.8 million tonnes (Mt) of rice and 113.3 Mt of wheat. With productivity levels of 4,057 kg ha⁻¹ for rice and 3,533 kg ha⁻¹ for wheat (FAOSTAT, 2021; Dhanda *et al.*, 2022), These results demonstrate how the Green Revolution changed the socioeconomic circumstances of millions of farmers and increased India's production of cereals from 87.4 million tons in 1961 to 332 million tons in 2023–2024. However, sustainability issues are escalating for the RWCS, with persistent problems such as weed infestations, heavy machinery compaction, fertilization-induced imbalances, groundwater depletion, and inadequate crop residue management threatening long-term productivity and soil health (Kumar *et al.*, 2024). Among these, weed pressure remains one of the most formidable challenges, especially under zero tillage (ZT) and direct-seeded rice systems.

The adaptability of weeds to changing agronomic practices renders them highly competitive and difficult to manage. In dry direct-seeded rice, weed-related yield losses can reach 85–98%, while wheat under ZT may suffer up to 70% yield reductions (Mishra *et al.*, 2022). Weed species such as *Cyperus rotundus*, *Echinochloa spp.* and *Celosia argentea* in rice, and *Phalaris spp.*, *Chenopodium album* and *Kochia scoparia* in wheat, are among the most problematic (Singh *et al.*, 2012; Nakka *et al.*, 2019). A diverse array of weed management strategies has been implemented to address infestations in the Rice-Wheat Cropping System (RWCS). These include mechanical methods such as hand weeding and tillage; cultural practices like crop rotation, delayed sowing, residue mulching, and the use of competitive cultivars; physical approaches such as soil solarization; as well as biological and chemical methods. However, the resilience and adaptability of weed species have often limited the long-term effectiveness of these approaches, particularly under intensive cropping systems. As a result, chemical herbicides have emerged as the primary tool for weed management. Factors such as rising labor costs and labor shortages during peak agricultural periods have further contributed to the growing reliance on herbicides, making them a more attractive and cost-effective option. Currently, approximately 57% of the wheat area under RWCS is managed using herbicides

Although herbicides are highly efficacious in controlling weeds, their widespread and often astronomical applications has raised significant ecological concerns, especially regarding their impact on agriculturally beneficial microorganisms (ABMs). These microorganisms, which include mycorrhizal fungi, phosphate-solubilizing bacteria (like *Pseudomonas* and *Bacillus*), nitrogen-fixing bacteria (like *Rhizobium* and *Azotobacter*), and biocontrol agents (like *Trichoderma* and *Bacillus subtilis*), are essential for nutrient cycling, preserving soil fertility, and fostering plant health. Disruption of these microbial communities can undermine the long-term sustainability of

agricultural systems. These microbes enhance nutrient availability, produce growth-promoting hormones, suppress pathogens, and improve plant resilience against environmental stresses. However, herbicide overuse, estimated to represent 42% and 32% of total herbicide use in wheat and rice, respectively, can disrupt microbial diversity and function, undermining the ecological foundation of sustainable agriculture (Pattanayak *et al.*, 2022).

Research indicates that over 98% of insecticides and 95% of herbicides affect soil microorganisms that are not their intended target, severely affecting microbial biodiversity and soil enzyme activities essential for nutrient cycling. Significant impairments are made to processes such as microbial carbon biomass, phosphorus solubilization, and nitrogen fixation, which lower crop production and soil fertility. Moreover, microbial functional diversity, an essential indicator of soil health, is significantly influenced by tillage practices, residue management, and herbicide applications. Specific herbicides have been found to inhibit key microbial activities, including phosphatase activity, nitrification, and beneficial microbial populations like *Pseudomonas* and mycorrhizal fungi. These disruptions can result in long-term declines in soil health, even if microbial biomass remains unchanged. In this context, it becomes essential to adopt integrated, ecologically sound weed management strategies that balance crop productivity with environmental stewardship.

This technical bulletin provides a comprehensive analysis of how chemical weed management practices affect agriculturally beneficial microorganisms and their ecological functions within the rice-wheat cropping system. It synthesizes current research on herbicide impacts on soil microbial diversity, function, and health, and presents findings from long-term field study conducted on the rice-wheat system. The study explores the interactions between weed control methods and soil microbiology under varying tillage regimes, offering practical insights for conservation agriculture.

The bulletin aims to promote the development and adoption of integrated and sustainable weed management strategies for crops such as rice, wheat, chickpea, and green gram. Emphasizing the judicious and efficient use of herbicides, it highlights practices that enhance weed control while safeguarding soil fertility and supporting beneficial soil microbial communities. These findings are intended to guide researchers, extension workers, and policymakers in promoting ecologically balanced, high-yielding farming systems.

02

Farmer's Perception of Herbicide Usage

Increasing agricultural productivity while reducing production costs and maintaining long-term soil health presents a major challenge for modern agriculture. Farmers today are becoming more aware of the crucial role soil plays in sustaining crop yields and providing essential ecosystem services. This growing awareness has influenced their perceptions regarding the use of herbicides in crop management, particularly within intensive systems like the rice-wheat rotation. Farmers' perceptions of herbicide usage varies and depends on specific contexts. Many farmers acknowledge the benefits of herbicides, particularly in terms of labor savings and weed control efficiency, but there is also considerable concern about their long-term impacts on soil health and the environment. In a study, 80–90% of respondents reported that continuous herbicide application over several years led to noticeable deterioration in soil structure, including cracking and hardening. Some farmers observed that prolonged herbicide use made soils less suitable for crop growth and, paradoxically, encouraged the proliferation of invasive weed species (Banjo *et al.*, 2010).

Farmers also express environmental concerns, echoing broader global worries about herbicide residues in crop produce, groundwater, and soils. Prior research supports these concerns, indicating that herbicides can negatively affect soil enzymatic activities and microbial functions, critical components of soil health and fertility (Bhardwaj *et al.*, 2021; Rose *et al.*, 2016). For instance, disruptions to earthworm populations and increased susceptibility to soil-borne diseases have been reported in fields exposed to common herbicides like glyphosate and atrazine. According to a study, a significant portion of farmers, particularly in states like Punjab, Haryana, and Andhra Pradesh, regard herbicides as indispensable, with 60–70% of farmers in these regions viewing them as essential inputs to reduce labor costs in light of rising wage rates. Moreover, over 75% of surveyed farmers cited time-saving as a key benefit, enabling timely weed control during labor-scarce peak agricultural seasons. Additionally, farmers favored broad-spectrum herbicides due to their effectiveness against a wide range of perennial weeds (Islam *et al.*, 2024).

A survey of 412 randomly chosen farmers was carried out across diverse agro-climatic zones of India to assess their knowledge and awareness of weed management technologies. Factor analysis of the data revealed that perceived risks associated with herbicide use were among the primary barriers to acceptance of weed management methods based on herbicides. Farmers expressed concerns about the possible harm that herbicides could do to the environment, crop yields, and soil health, which influenced their decision-making process (Gharde and Singh, 2021). These findings align with those of earlier studies, such as Nath *et al.* (2024), which also identified comparable barriers to herbicide adoption. The results accentuate the critical need to strengthen farmer education and extension services, particularly in areas related to herbicide safety, precise



application techniques, and the promotion of integrated weed management practices. Educating farmers on the ecological implications of herbicide use and promoting sustainable, long-term weed management practices are crucial steps toward ensuring the health of the soil and maintaining the yield potential of the rice-wheat cropping system. Striking a balance between achieving immediate weed control and safeguarding long-term soil fertility remains a vital consideration in the widespread adoption of herbicides in agricultural systems. Enhancing farmers' knowledge and awareness is crucial for promoting the safe and efficient application of herbicides. This will help ensure both immediate productivity gains and long-term ecological sustainability. Achieving a balance between short-term benefits and long-term soil health is a critical priority in the adoption and use of herbicides, especially in rice-wheat cropping systems.

03

Chemical Weed Management in Rice-Wheat Cropping System

Weed management using chemicals (herbicides) plays a critical role in sustaining productivity in the Rice-Wheat Cropping System (RWCS), which is widely practiced across South Asia. Driven by labor scarcity, escalating wages, and time constraints during critical farming periods, herbicides have become the preferred weed management option among farmers. Herbicides offer quick and broad-spectrum weed suppression, particularly under conservation agriculture practices like zero tillage (ZT) and direct-seeded rice (DSR), where mechanical weed control options are limited (Kumar *et al.*, 2024; Mishra *et al.*, 2022).

Herbicides used in the RWCS include both pre-emergence agents such as pendimethalin and pretilachlor, and post-emergence herbicides like bispyribac-sodium and metsulfuron-methyl. These chemicals are effective against a wide range of grassy and broadleaf weeds, which are otherwise difficult to manage manually or mechanically. However, the widespread use of herbicides has raised agronomic and ecological concerns, including weed resistance, alterations in weed flora, and adverse effects on soil health and beneficial microorganisms (Nakka *et al.*, 2019; Pattanayak *et al.*, 2022). In India, herbicides currently account for about 12% of total agrochemical usage, compared to 43% globally and over 55% in the United States (Gharde and Singh, 2021). While herbicides are cost-effective and labor-saving, their overuse can degrade soil quality by affecting enzymatic activity, microbial biomass, and nutrient cycling. Some of the major herbicides used in rice and wheat cropping are outlined in Table 1 (Choudhary and Dixit, 2018; Choudhury *et al.*, 2016; Dixit & Varshney, 2009).

Table 1: List of common herbicides used in different cropping pattern in rice-wheat cropping system

Herbicide Name	Formulation	Dose (a.i. ha ⁻¹)	Application Timing
RICE			
Pre-emergence			
Bensulfuron-methyl	60% DF	0.06	3 DAT
Butachlor	50% EC	1.0–1.5	2–3 DAT
Oxyfluorfen	23.5% EC	0.15–0.20	1–3 DAS (DSR)
Paraquat dichloride	24% SL	0.30–0.80	Pre-plant
Pendimethalin	38.7% CS	0.678	1–3 DAS (DSR)
Pendimethalin	30% EC	1.0–1.5	0–5 DAS
Pretilachlor	37% EW / 30.7% EC	0.45–0.75	6–7 DAT
Pretilachlor + Safener	50% EC	0.5–0.75	0–5 DAT

<i>Post-emergence</i>			
2,4-D Sodium Salt	80% WP /	0.5–1.0	25–35 DAS
2,4-D Amine Salt	58% SL	0.40–0.50	25–35 DAS
Anilofos	30% EC	0.30–0.45	3–5 DAT
Bispyribac-sodium	10% SC	0.025	15–25 DAS
Cyhalofop-butyl	10% EC / 5% EC	0.075–0.10	15–25 DAS
Ethoxysulfuron	15% WP	0.015–0.018	15–25 DAS
Fenoxaprop-p-ethyl	9% EC	0.05–0.06	25–30 DAS
Metsulfuron-methyl + Chlorimuron ethyl	10% + 10% WP	0.004	25–35 DAT
Penoxsulam	21.7% SC	0.020–0.025	10–20 DAS
Pretilachlor + Pyrazosulfuron	6% GR	0.615	0–5 DAT
Pyrazosulfuron-ethyl	10% WP	0.010–0.015	8–10 DAT
Triafamone	20% WG	0.025–0.030	20–20 DAT
<i>WHEAT</i>			
<i>Pre-emergence</i>			
Flufenacet + Metribuzin	44.4% SE	0.30–0.35	0–3 DAS
Pendimethalin	30% EC	0.75–1.0	0–3 DAS
<i>Post-emergence</i>			
2,4-D Sodium Salt	80% WP	0.5–1.0	30–35 DAS
2,4-D Amine Salt	58% SL	0.40–0.50	30–35 DAS
Pyroxasulfone	84 % WG	0.127	0–3 DAS
Carfentrazone-ethyl	40% DF	0.020–0.025	25–35 DAS
Clodinafop + Metsulfuron-methyl	15% + 1% WP	0.060 + 0.004	25–30 DAS
Clodinafop-propargyl	15% WP	0.06	25–30 DAS
Fenoxaprop + Metribuzin	9% + 20% WP	0.054 + 0.12	20–30 DAS
Isoproturon	75% WP	0.75–1.0	25–30 DAS
Mesosulfuron + Iodosulfuron	3.6% + 0.9% WG	0.012 + 0.0024	25–30 DAS
Metribuzin	70% WP	0.20–0.30	20–30 DAS
Metsulfuron-methyl	20% WP	0.004	25–30 DAS
Pinoxaden	5.1% EC	0.05–0.06	25–30 DAS
Sulfosulfuron	75% WG	0.025	20–25 DAS
Sulfosulfuron + Metsulfuron	75% WG	0.030 + 0.002	20–25 DAS

04

Effect of Herbicides on Agriculturally Beneficial Soil Microorganisms

The use of chemical weed management practices, particularly herbicides, has been a cornerstone of modern agriculture due to their efficacy in controlling weed populations. However, these chemicals may have unintended consequences on non-target soil organisms, particularly agriculturally beneficial microorganisms that aid in the nutrient cycling, plant growth promotion, and disease suppression. The severity of the impact is contingent upon a range of variables, including the type of herbicide, its concentration, the duration of exposure, and the native composition of the soil microbiota. Understanding the interplay between herbicides and beneficial soil microbes is critical for preserving soil health and promoting resilient agricultural systems (Barman & Varshney, 2008).

4.1. Mycorrhizal Fungi

Vesicular-Arbuscular Mycorrhizae (VAM), a key group of mycorrhizal fungi, form symbiotic relationships with plant roots that significantly enhance phosphorus uptake. Herbicide interference with these associations may impair agroecosystem health and functionality. In a study conducted at the Directorate, the effects of post-emergent herbicides, clodinafop, sulfosulfuron, and pinoxaden (applied alone or with a surfactant, A12127) - were evaluated on VAM colonization in wheat roots (DWR AR, 2007). Results indicated a dose-dependent decline in VAM colonization with increasing concentrations of pinoxaden, with a 19% greater reduction when combined with the surfactant. Clodinafop, even at the recommended dose, proved more toxic to VAM than pinoxaden, whereas sulfosulfuron had no adverse impact, suggesting it as a safer alternative for maintaining VAM-root associations (Fig. 1).

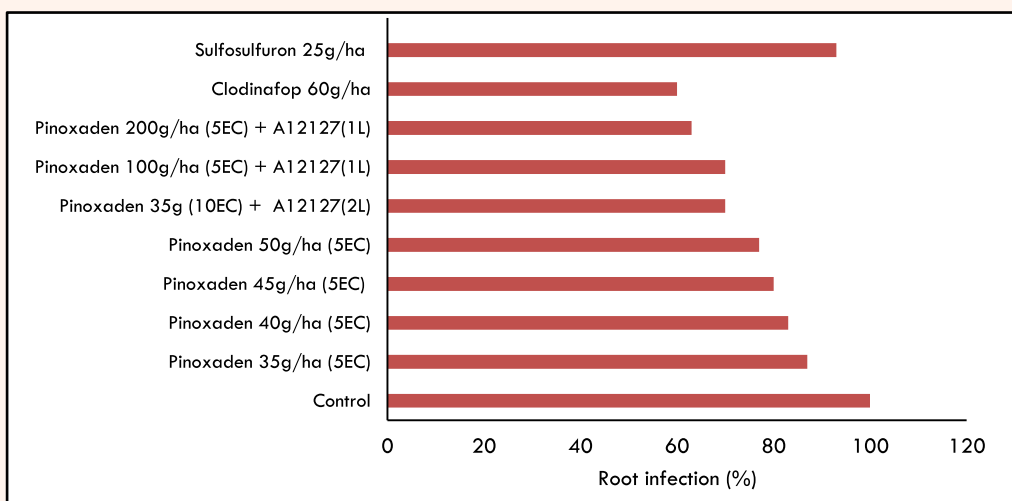


Figure 1: Effect of pinoxaden, clodinafop, and sulfosulfuron on wheat root infection by VAM

Another study (DWR AR, 2006) assessed the effect of multiple herbicides—buthachlor, anilophos, isoproturon, sulfosulfuron, clodinafop, and 2, 4-D, on VAM in sorghum roots at three concentrations (1X, 5X, and 10X). Buthachlor only reduced VAM colonization and spore density at 5X, while sulfosulfuron reduced spore density at the recommended dose and root colonization only at higher doses. Conversely, anilophos, isoproturon, clodinafop, and 2, 4-D significantly reduced both VAM root infection and spore density even at the lowest concentration. Clodinafop was the most toxic, followed by 2,4-D, isoproturon, anilophos, sulfosulfuron, and butachlor (Fig. 2).

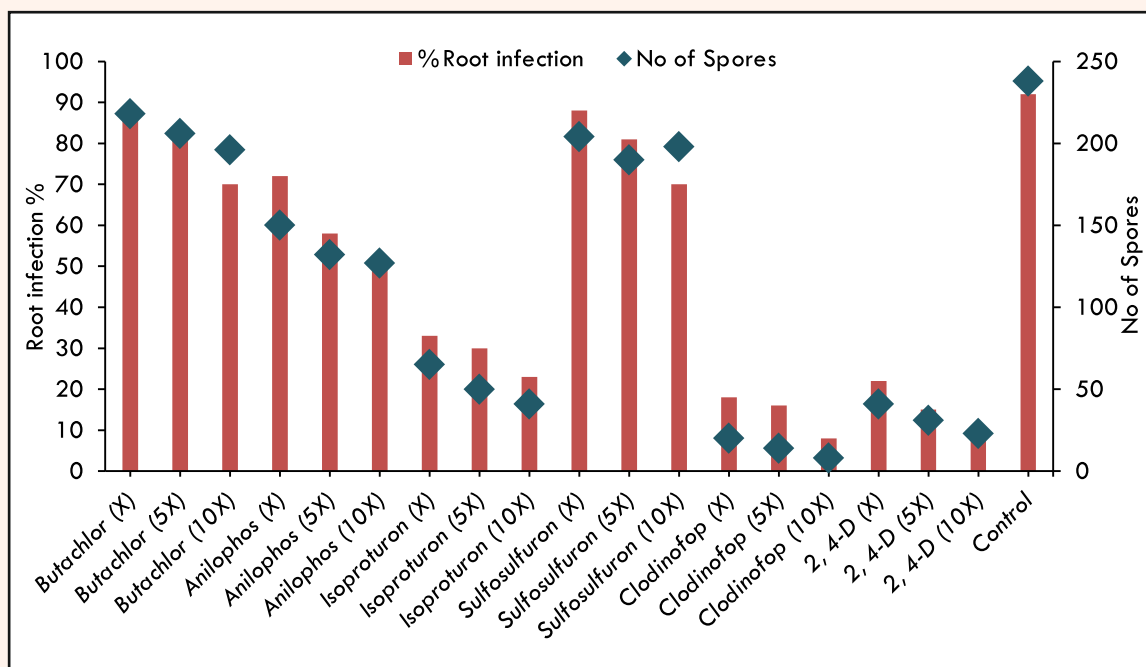


Figure 2: Dose-dependent effect of different herbicides on VAM colonization in wheat roots

These studies demonstrate that herbicide effects on VAM associations in wheat systems vary by compound and concentration. Clodinafop, 2,4-D (especially its ethyl ester form), and high doses or combinations of pinoxaden significantly reduce mycorrhizal spore abundance and root colonization, likely due to their volatility, hormonal disruption, or non-target microbial toxicity (Zaller *et al.*, 2014). Clodinafop and pinoxaden, though designed to selectively target grass weeds, may exhibit non-target toxicity at higher doses, influencing microbial communities and mycorrhizal functioning indirectly via alterations in root exudate composition or soil microbial dynamics. In contrast, sulfosulfuron shows minimal impact, possibly due to its narrow spectrum and lower soil persistence (Taibi *et al.*, 2021).

4.2. Nitrogen-fixing bacteria

Nitrogen-fixing bacteria, including diazotrophs and nitrifiers, enhance soil fertility by converting atmospheric and nitrogenous compounds into plant-available forms, improving nitrogen use efficiency and supporting sustainable crop productivity.

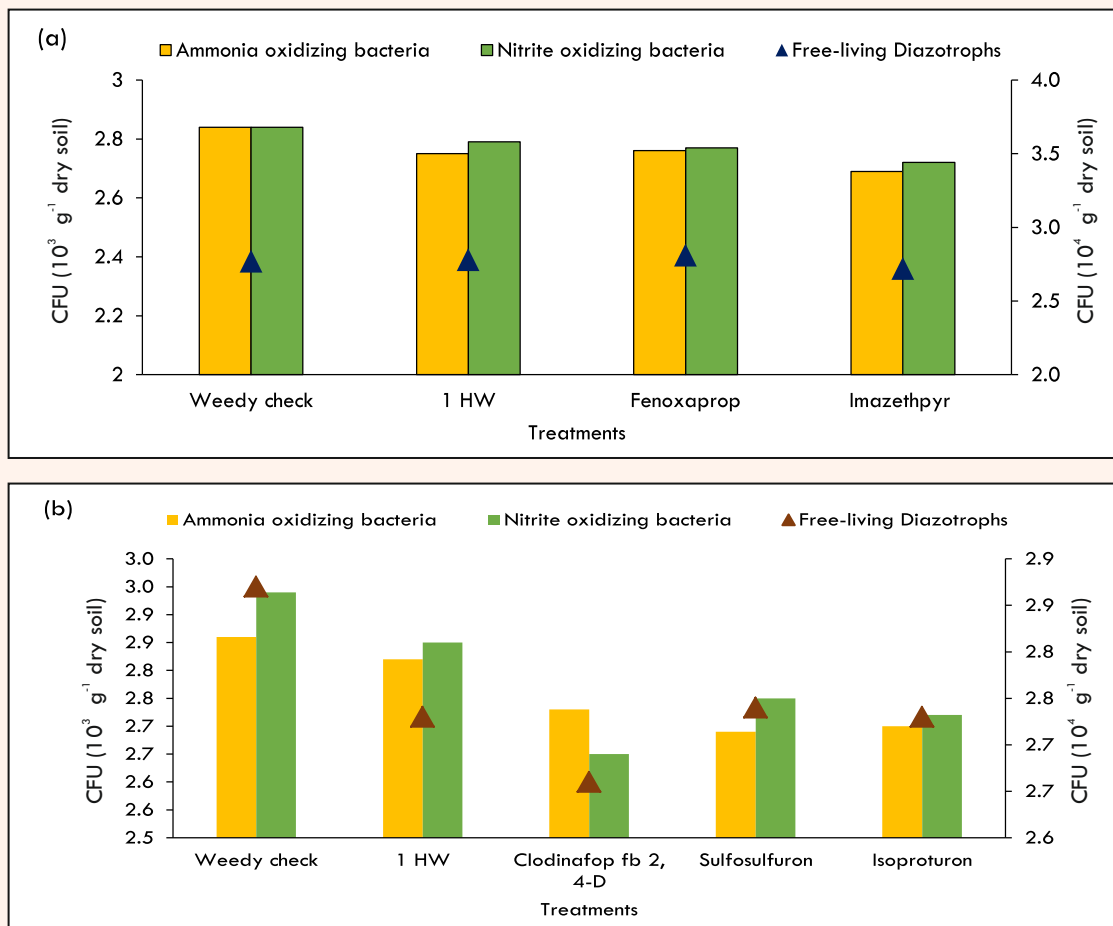


Figure 3: Effect of (a) fenoxaprop, imazethapyr, and (b) clodinafop fb 2,4-D, Sulfosulfuron, isoproturon on nitrogen fixing bacteria

An investigation (DWR AR, 2010) was carried out to assess the effects of different herbicides on nitrogen-fixing bacterial communities in soybean and wheat crops. Herbicide application significantly affected the abundance of these bacterial groups in both crops, indicating herbicide-specific influences on nitrogen-fixing microbial communities (Fig. 3). In soybeans, at the harvesting stage, the herbicide Fenoxaprop supported the highest populations of free-living diazotrophs ($3.08 \times 10^4 \text{ g}^{-1}$), ammonia oxidizers ($3.06 \times 10^3 \text{ g}^{-1}$), and nitrite oxidizers ($3.14 \times 10^3 \text{ g}^{-1}$), compared to Imazethapyr, which showed lower values for all three microbial groups (Fig. 3a). This suggests that Fenoxaprop is less detrimental to the nitrogen-fixing microbial community than Imazethapyr,

possibly due to differences in chemical composition, soil persistence, or microbial selectivity. In wheat, Sulfosulfuron treatment supported the highest populations of free-living diazotrophs ($2.74 \times 10^4 \text{ g}^{-1}$) and nitrite oxidizers ($2.75 \times 10^3 \text{ g}^{-1}$), while Clodinafop was most favorable for ammonia oxidizers ($2.73 \times 10^3 \text{ g}^{-1}$), indicating a crop- and herbicide-specific interaction with soil microbial communities (Fig. 3b). Across both crops, the treatments involving hand weeding and weedy check consistently maintained higher microbial populations compared to chemical herbicide applications. This pattern highlights the potentially adverse effects of herbicides on beneficial soil microorganisms involved in nitrogen cycling. Herbicide-induced microbial suppression may result from direct toxicity or indirect effects such as altered root exudation patterns and nutrient availability, as noted by Kremer and Means (2009). Furthermore, as ammonia and nitrite oxidizers play a crucial role in nitrification, any reduction in their populations could negatively affect soil nitrogen dynamics and crop nutrient availability.

4.3. Plant Growth-Promoting Rhizobacteria (PGPR)

Plant growth-promoting rhizobacteria (PGPR), particularly *Pseudomonas fluorescens*, serve as a cornerstone of sustainable agriculture through their ability to suppress soil-borne and foliar pathogens and enhance plant health. However, herbicide applications can significantly compromise their viability and function. An in vitro study evaluating the impact of eight herbicides at 0.5X, 1X, and 2X recommended doses on a *P. fluorescens* isolate from chickpea rhizosphere revealed a pronounced, dose-dependent suppression of bacterial populations (DWR AR, 2006). Herbicides such as oxyfluorfen, anilophos, metribuzin, isoproturon, and pendimethalin were particularly toxic, causing 91–97% reduction in bacterial colony-forming units (c.f.u.) at 2X doses. In contrast, butachlor exhibited relatively low toxicity, especially at 0.5X concentration (Fig. 4). Herbicides such as oxyfluorfen, anilophos, metribuzin, isoproturon, and pendimethalin exhibit high toxicity toward PGPR like *Pseudomonas fluorescens* by disrupting membrane integrity, inducing oxidative stress, and interfering with key metabolic processes, ultimately reducing their viability and functional efficiency (Grossmann, 2010; Jacobsen & Hjelmsø, 2014).

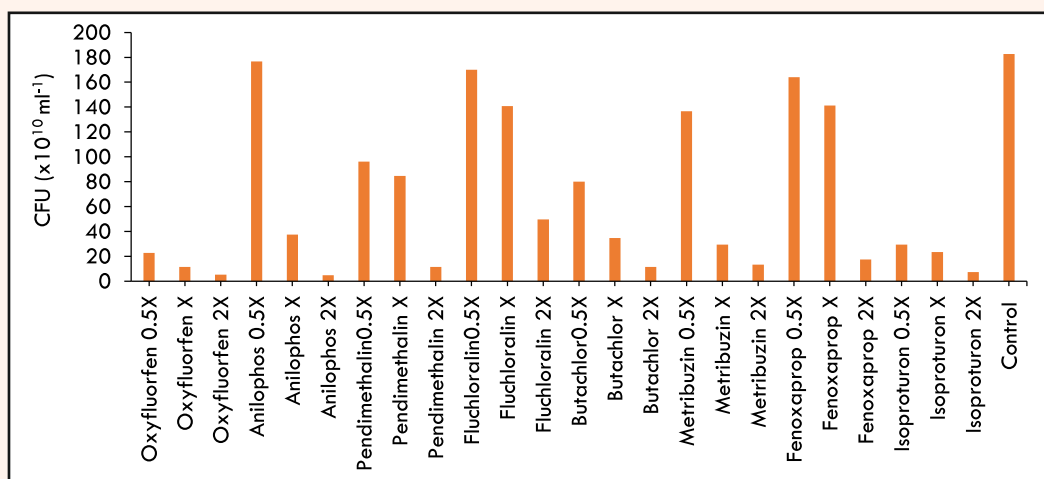


Figure 4: Effect of different herbicides with varying doses on plant growth-promoting bacteria, *Pseudomonas fluorescens*

4.4. Phosphate-Solubilizing Fungi (PSF)

Phosphate-solubilizing fungi (PSF), including *Aspergillus*, *Penicillium*, and *Trichoderma*, are crucial for enhancing phosphorus availability in the soil. A study (DWR AR, 2006) evaluated the effects of six herbicides, butachlor, anilophos, isoproturon, sulfosulfuron, 2, 4-D, and clodinafop, on these fungi. The results indicated that *Aspergillus* exhibited the highest sensitivity to herbicidal treatments, with negative effects from all except sulfosulfuron, which could disrupt phosphorus cycling and soil fertility (Rawat *et al.*, 2021). On the other hand, *Trichoderma* exhibited moderate sensitivity, particularly to isoproturon and clodinafop, both of which are known to impact soil microbial communities (Bharadwaj *et al.*, 2024). *Penicillium* showed remarkable resistance, with no observable toxicity from any of the herbicides tested, suggesting that some PSF may be more resilient to chemical stress than others. Sulfosulfuron was the least toxic, suggesting its potential for use in herbicide regimes that protect soil microorganisms (Pandey *et al.*, 2006).

4.5. Cellulose-degrading bacteria

In a study conducted under conservation agriculture (DWR AR, 2023), the effects of different herbicides and their combinations on cellulose-degrading bacterial (CB) populations were assessed. At 45 days after sowing (DAS), zero tillage (ZT) treatments exhibited approximately twofold higher CB populations compared to conventional tillage (CT) across all weed management practices (Fig. 5). Although herbicide application significantly reduced CB populations, the decline was less pronounced under ZT than CT. This indicates that ZT may provide a more conducive environment for cellulose-degrading bacteria, likely due to reduced soil disturbance, greater organic matter retention, and enhanced microbial habitat quality (Lupwayi *et al.*, 2018). Among the treatments, W1-ZT recorded the highest CB population, whereas the application of pretilachlor + pyrazosulfuron followed by bispyribac sodium resulted in a marked decline in bacterial abundance.

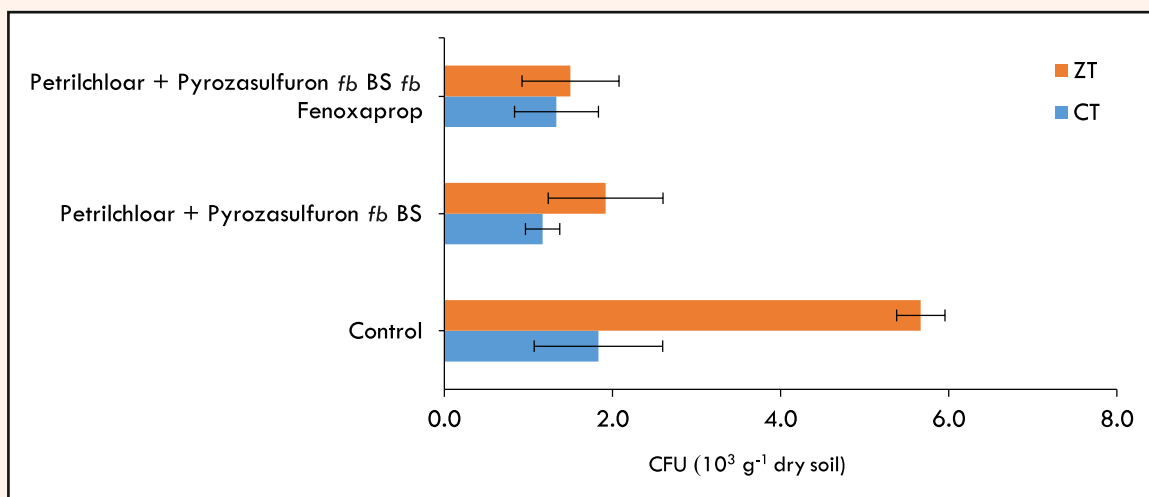


Figure 5: Effect of herbicides and tillage practices on cellulolytic bacterial population at 45 days after sowing. BS: Bispyribac Sodium

4.6. Biocontrol Agents

Trichoderma species are well-established fungal biocontrol agents effective against soil-borne pathogens. A study (DWR AR, 2005) evaluated the impact of various soil-applied herbicides on *Trichoderma* mycelial biomass after 10 days of incubation. Alachlor, at the recommended dose (2 ppm), produced the highest *Trichoderma* biomass, followed closely by the half-dose (0.5X), while simultaneously suppressing *Sclerotium rolsii* at both recommended and double doses. Fluchloralin strongly inhibited *S. rolsii* with moderate effects on *Trichoderma*, indicating its potential as a selective input for integrated weed and disease management. In contrast, metribuzin significantly reduced *Trichoderma* biomass across all concentrations while supporting relatively higher *S. rolsii* growth, second only to the untreated control. The broad-spectrum toxicity and persistence of metribuzin, a triazinone herbicide known to inhibit photosystem II and disrupt fungal metabolism (Streletsii *et al.*, 2023), may account for its negative impact on beneficial fungi.

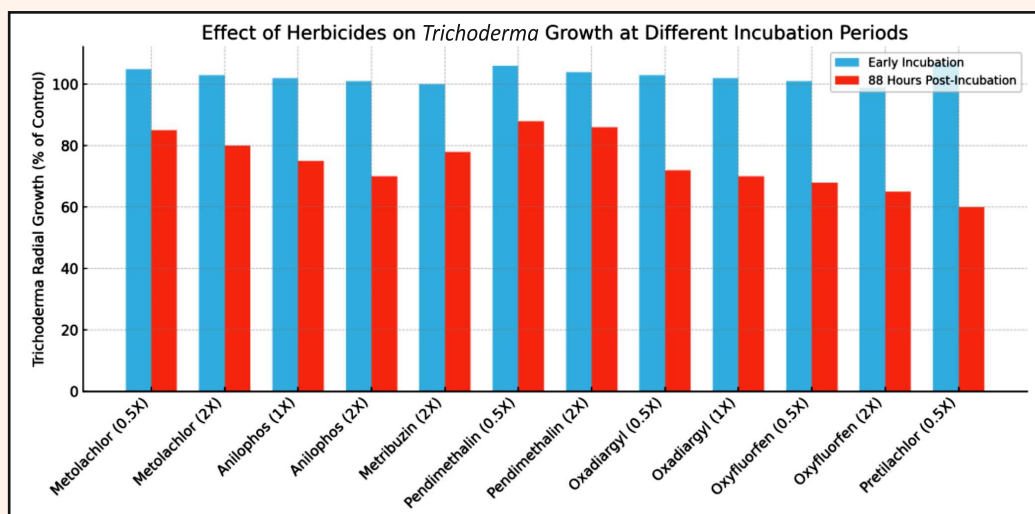


Figure 6: Dose-dependent effect of herbicides on radial growth of *Trichoderma*

A complementary laboratory study assessed the influence of pre-emergence and pre-plant incorporated herbicides on *Trichoderma* (BMIR isolate) using the dual culture technique. Early incubation showed slight stimulation of *Trichoderma* radial growth by several herbicides, including metolachlor (0.5X–2X), anilophos (X, 2X), metribuzin (2X), pendimethalin (0.5X, 2X), oxadiargyl (0.5X, X), oxyfluorfen (0.5X, 2X), and even at the lowest dose of pretilachlor (0.5X). However, by 88 hours, all herbicides significantly suppressed *Trichoderma* growth compared to untreated controls (Fig. 6). Among them, pendimethalin (0.5X, 2X), metribuzin (2X), and metolachlor (0.5X) exhibited the least suppressive effects, suggesting their potential compatibility with *Trichoderma*. These findings highlight that selective herbicide use, particularly at lower doses, may enable concurrent weed and pathogen suppression without severely compromising biocontrol efficacy.

05

Effect of Herbicides on Major Soil Ecological Functions

5.1. Biological Nitrogen Fixation via Legume-Rhizobium Symbiosis

Biological nitrogen fixation (BNF), driven by the legume-*Rhizobium* symbiosis, underpins sustainable agriculture, especially in cereal-based systems like rice-wheat. In legumes such as soybean, *Rhizobium* species establish symbiosis in root nodules, where they convert inert atmospheric nitrogen (N_2) into plant-accessible forms (NH_3), that enhances soil fertility and crop productivity. Nodulation is a pivotal process in BNF, enabling effective symbiosis and nitrogen assimilation. However, herbicide application may impair this process by disrupting rhizobial populations, altering root exudation patterns, or interfering with plant hormone signaling, ultimately reducing nitrogen fixation efficiency and legume growth.

5.1.1. Nodulation in soybean

Studies were carried out to examine the effects of different herbicides applied for weed management on nodulation characteristics in soybean. These included field trials carried out during the Kharif seasons of 2009 and 2010 (DWR AR, 2010), along with controlled pot studies conducted in 2007 (DWR AR, 2008). The cumulative effects of different herbicides on soybean nodulation are discussed below:

5.1.1.1. Quizalofop, Fenoxaprop, and Imazethapyr

In a field experiment conducted at ICAR-DWR, compared to HW, adverse effects of herbicides was recorded in soybean nodulation. At 40 DAS, hand weeding exhibited the highest nodule count, followed by quizalofop (111.6%), fenoxaprop (95.8%), and imazethapyr (88.9%), compared to the control. The weedy control had the lowest count (18.9). At 60 DAS, HW still had the highest nodules (75.7), whereas herbicides like fenoxaprop, imazethapyr, and quizalofop showed 79.3%, 62.2%, and 59.0% increases over the weedy control, respectively (Table 2). The dry biomass accumulation at 40 DAS increased by 107.3% with HW, 105.4% with Fenoxaprop, 103.2% with Quizalofop, and 73.3% with Imazethapyr compared to the weedy control. At 60 DAS, the biomass increased by 69.7% with HW, 38.5% with Imazethapyr, 38.4% with Fenoxaprop, and 35.2% with Quizalofop compared to the weedy control. The root volume at 40 DAS was highest in quizalofop, followed by HW, with increases of 52.6% and 47.4%, respectively. Fenoxaprop and Imazethapyr had lower root volumes, with increases of 36.8% and 31.6%, respectively, compared to the weedy control.

Table 2: Effect of Quizalofop, Fenoxaprop, and Imazethapyr on nodulation characteristics in soybean

Treatment	Nodule count (Nos. plant ⁻¹)		Nodule Dry Biomass (mg plant ⁻¹)		Root Volume (cm ³ plant ⁻¹)	
	40 DAS	60 DAS	40 DAS	60 DAS	40 DAS	60 DAS
Quizalofop	40.0	35.3	268	240	2.9	3.9
Fenoxaprop	37.0	39.8	271	246	2.6	3.3
Imazethapyr	35.7	36.0	229	246	2.5	3.5
HW	56.1	75.7	278	301	2.8	3.9
Weedy	18.9	22.2	132	177	1.9	2.8

(Source: DWR AR 2007, 2009, 2010)

At 60 DAS, HW and Quizalofop showed the highest root volume, with increases of 39.3% over the control, followed by Imazethapyr (25.0%) and Fenoxaprop (17.9%) over the control (Table 2). There is a positive correlation between nodule count, nodule dry biomass, and root volume based on the treatments. Treatments like HW, which lead to higher nodule count and dry biomass, also promote better root development, suggesting that these traits may be linked in their response to treatment. Overall, hand weeding consistently outperformed all herbicide treatments. Among herbicides, Fenoxaprop showed a relatively better balance across all parameters at both 40 and 60 DAS. Quizalofop was effective at early stages (40 DAS), while Imazethapyr performed comparatively better by 60 DAS in terms of dry biomass and root volume. The weedy control significantly hindered nodulation and root development, confirming the importance of weed management for optimizing soybean root health and symbiotic nitrogen fixation

5.1.1.2 Clodinafop, Metribuzin, and UPH

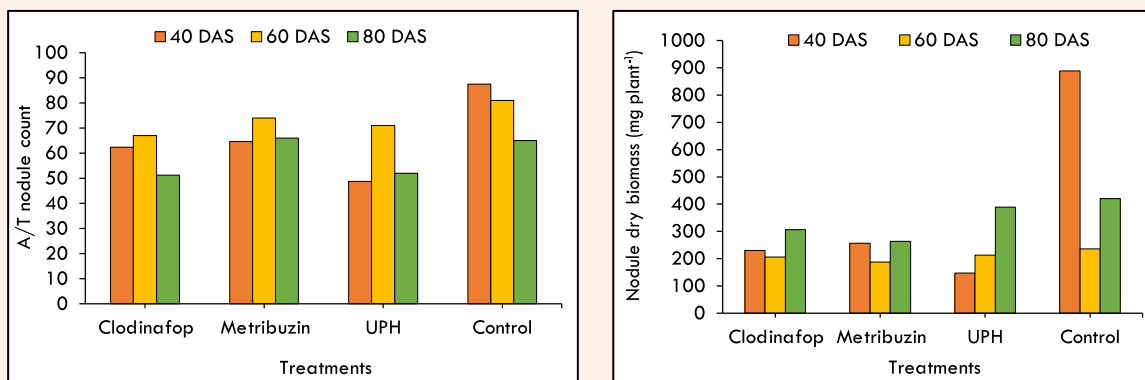


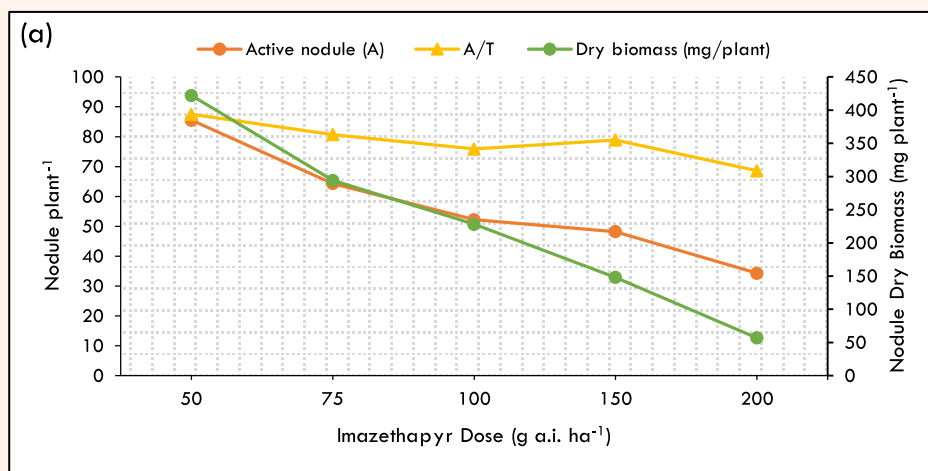
Figure 7: Effect of Clodinafop, Metribuzin, and UPH on (a) A/T ratio of nodule numbers, and (b) nodule dry biomass at different growth stages in soybean (Source: DWR AR 2007)

At 40 DAS, the active nodule to total nodule ratio (A/T) was highest in the control (no herbicide), whereas Clodinafop, Metribuzin, and UPH showed 28.4, 26.1, and 45.4% reduction compared to the control. Even at 60 DAS, control showed the highest nodule count. Among herbicides, metribuzin, clodinafop, and UPH resulted in 17.1, 8.5, and 13.4 % decline compared to the control. At 80 DAS, nodule counts declined across all treatments. Compared to the control, Clodinafop and UPH showed reductions of 21.5% and 20.0%, respectively, while Metribuzin slightly exceeded the control by 1.5% (Fig. 7a). Overall, the control consistently exhibited the highest nodule counts across all stages. Metribuzin showed the least suppressive effect on nodulation, particularly at 80 DAS, where it even slightly improved nodulation. In contrast, UPH and Clodinafop had more substantial negative effects, especially at 40 DAS, indicating early-stage sensitivity of nodulation to these herbicides. The data suggest that Metribuzin is relatively safer for nodule formation compared to Clodinafop and UPH.

Application of Clodinafop, Metribuzin, and UPH resulted in significant reductions in nodule dry biomass at early growth stages (40 DAS) compared to the untreated control, with Clodinafop and UPH showing the highest reductions of approximately 75.6% and 81.1%, respectively (Fig. 7b). At 60 DAS, the negative impact of these herbicides persisted but was less severe, with reductions ranging from 23.3% to 33.3%. By 80 DAS, the differences narrowed considerably. Notably, UPH showed a 14.3% increase over the control, indicating recovery or compensatory growth, while Clodinafop and Metribuzin still showed slight reductions. These results suggest that while all three treatments suppress early nodulation, UPH may support late-stage nodulation recovery better than Clodinafop or Metribuzin.

5.1.1.3. Dose-dependent effects of imazethapyr and quizalofop

The dose-dependent effects of imazethapyr and quizalofop on nodulation parameters in soybean reveal a clear trend of herbicide-induced suppression of symbiotic development.



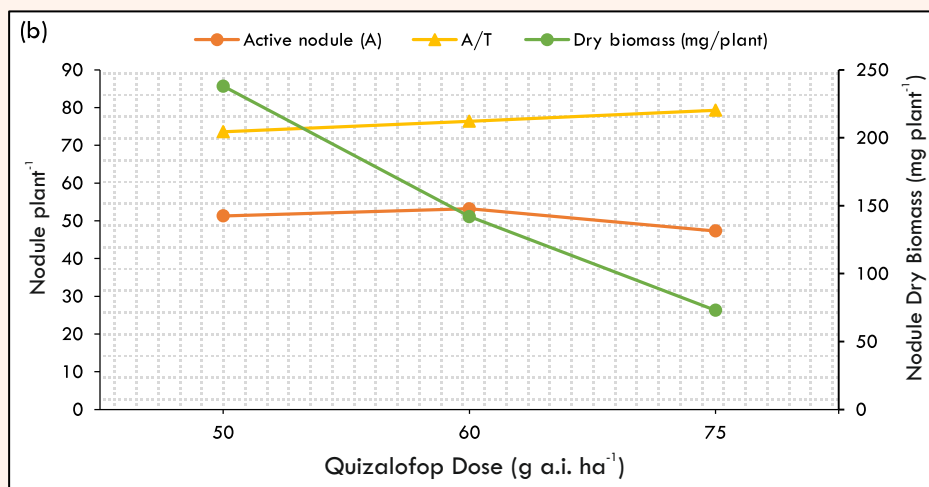


Figure 8: Dose-dependent effects of (a) imazethapyr and (b) quizalofop on nodulation parameters in soybean (Source: DWR AR 2007)

Increasing doses of imazethapyr progressively reduced total and active nodule counts, nodule dry weight per plant, and unit dry weight per nodule. Compared to the control, total nodule count decreased by 14.7% at 50 g a.i. ha⁻¹ and by 56.1% at 200 g a.i. ha⁻¹ (Fig. 8a). Similarly, active nodules declined by 19.5% to 67.7%, and total nodule dry weight per plant fell drastically from 780 mg in the control to just 57 mg at the highest imazethapyr dose, a 92.7% reduction. The unit dry weight per nodule also dropped from 6.9 mg to 1.2 mg, indicating impaired nodular development. A steady, dose-dependent decline in both active nodules and nodule dry matter. At the highest dose (200 g ha⁻¹), nodulation parameters dropped by over 80%, highlighting strong phytotoxicity.

Quizalofop exhibited a similar but slightly less severe impact. Total nodule counts with quizalofop ranged from 70.3 to 59.7, representing a 38.6–47.8% decrease over control, while active nodules declined by 49.9–55.7% (Fig. 8b). Nodule biomass per plant was reduced by 69.5–90.6%, and unit dry weight dropped from 6.9 mg in the control to as low as 1.2 mg at 75 g a.i. ha⁻¹. These findings suggest that while both herbicides significantly inhibit nodulation, imazethapyr poses a greater risk to rhizobial activity and nodule function. The control and weedy plots maintained the highest nodulation values, underscoring the detrimental effect of these post-emergence herbicides on biological nitrogen fixation in soybean.

5.1.2. Nodulation in Chickpea and Pea

Field experiments conducted during the rabi seasons of 2009–10 and 2022 evaluated the impact of chemical weed control practices on nodulation and biological nitrogen fixation in chickpea (*Cicer arietinum* cv. JG-16) and field pea (*Pisum sativum* cv. JP-885) (DWR AR, 2010 and 2022). These studies primarily assessed how herbicides such as fluchloralin, pendimethalin, clodinafop, quizalofop, and certain combinations, including imazethapyr and topamezone, influenced nodule formation, rhizobial activity, weed suppression, and ultimately seed yield.

In chickpea, the application of fluchloralin (1 kg ha⁻¹ PPI) and pendimethalin (1 kg/ha PE) significantly reduced nodule count and dry weight at 40 and 60 days after sowing (DAS) compared to hand-weeding (Fig. 9). However, these negative effects were transient, with nodulation recovering by 90 DAS. Although pendimethalin provided effective weed control, comparable to hand weeding, and contributed to similar seed yields, its adverse impact on nodulation during the peak vegetative phase raised concerns about its compatibility with sustainable nitrogen fixation.

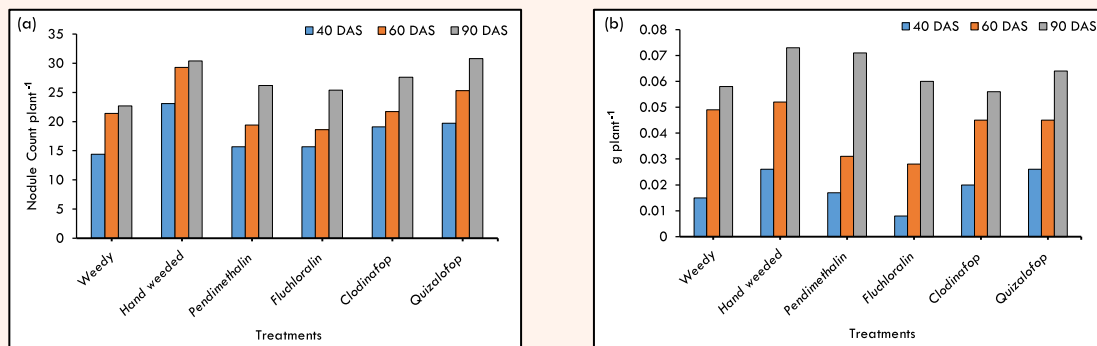


Figure 9: Effect of pendimethalin, fluchloralin, clodinafop, and quizalofop on (a) nodule count and (b) nodule dry weight in chickpea (Source: DWR AR 2010)

Follow-up investigations in 2022 revealed that weed competition and herbicide combinations further influenced the rhizosphere environment (DWR AR, 2023). The lowest rhizobia population and nodule biomass were observed in the unweeded plots, underscoring the adverse impact of weed competition on root nodulation. However, the treatment Pendimethalin *fb* Topramezone showed a clear suppressive effect on nodule weight and rhizobial population, indicating toxicity of this herbicide combination to the symbiotic system (Fig. 10).

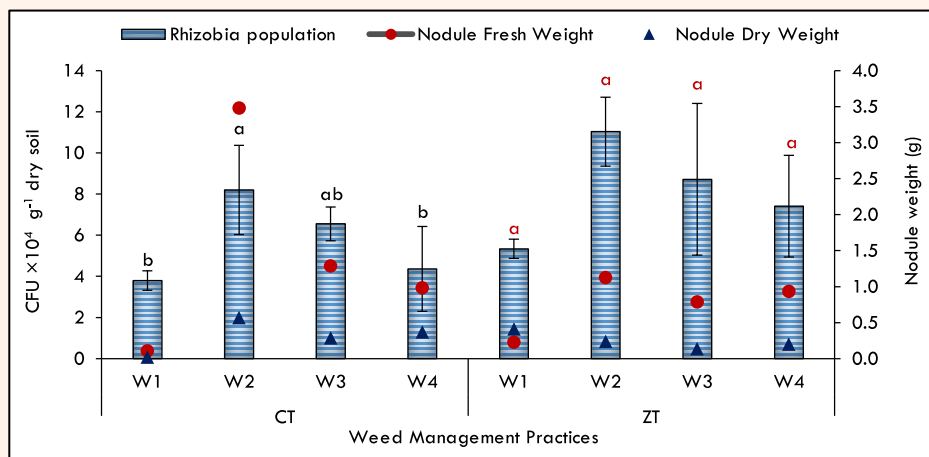


Figure 10: Effect of different chemical weed management and tillage practices on root nodule dry and fresh weight, and Rhizobia population. W1: Control; W2: Pendimethalin + Imazethapyr *fb* Topramezone; W3: Pendimethalin *fb* Hand weeding; W4: Pendimethalin *fb* Topramezone. CT: Conventional Tillage, ZT: Zero Tillage (Source: DWR AR 2022)

A similar experiment was conducted on field pea (*Pisum sativum*, cultivar JP-885) during the same season (2009–10), using the same herbicide treatments and their effect on nodule characters was analyzed 65 days after sowing (DWR AR, 2010). Among these, Fluchloralin significantly reduced nodule count compared to hand weeding, indicating phytotoxicity. Pendimethalin, clodinafop, and quizalofop showed moderate effects, with nodule counts statistically similar to the untreated (weedy) plots. In terms of weed control, all herbicide treatments (except the weedy control) substantially reduced weed dry matter (Table 3). However, seed yield was significantly lower in pendimethalin-treated plots compared to hand-weeding, suggesting that despite effective weed suppression, the herbicide's adverse effect on nitrogen fixation limited crop productivity.

Table 3: Effect of pendimethalin, fluchloralin, clodinafop, and quizalofop on weed infestation, nodulation, and seed yield production in pea

Treatments	Weed dry matter (g m ⁻²)	Nodule count plant ⁻¹	Seed yield (t ha ⁻¹)
Weedy	10.0	29.1	2.09
Hand weeded	3.9	35.1	2.27
Pendimethalin	5.4	29.5	1.79
Fluchloralin	4.3	25.5	1.94
Clodinafop	7.0	29.9	2.09
Quizalofop	4.8	30.3	2.13
LSD (0.05)	1.5	9.3	0.35

(Source: DWR AR 2010)

5.1.3. Nodulation in Blackgram

According to DWR AR (2012), all herbicide treatments had a negative effect on nodule count and nodule dry biomass output in blackgram (var. T-9) as compared to hand weeding. Under the weedy check, the nodule count and dry biomass production were the lowest.

Table 4: Effect of Pendimethalin, clodinafop, and quizalofop on nodulation in Blackgram

Treatments	Nodule count (No. plant ⁻¹)	Nodule dry biomass (mg plant ⁻¹)
Pendimethalin	80	64
Clodinafop	93	59
Quizalofop	74	89
Weed free	128	122
Weedy	51	36
LSD (0.05)	31	31

(Source: DWR AR 2012)

The data indicate that hand weeding (weed-free treatment) was the most effective in promoting root nodulation, with the highest nodule count (128 nodules plant⁻¹) and nodule dry biomass (122 mg plant⁻¹) (Table 4). Compared to this, Pendimethalin treatment resulted in a 37.5% reduction in nodule count and a 47.5% reduction in nodule biomass. Clodinafop caused a 27.3% decline in nodule count and a 51.6% decline in biomass. Quizalofop led to the greatest reduction in nodule count (42.2%) among herbicides but interestingly recorded a 27.0% increase in nodule biomass, suggesting fewer but possibly larger or more efficient nodules. The untreated weedy condition had the most detrimental effect, reducing nodule count by 60.2% and nodule biomass by 70.5% compared to hand weeding. These results highlight that although herbicide use generally suppressed nodulation compared to hand weeding, Quizalofop may support relatively better nodule development in biomass. On the other hand, weed infestation severely hampered both nodule formation and biomass accumulation.

5.1.4. Key Implications

The findings across multiple legume crops, including soybean (*Glycine max*), chickpea (*Cicer arietinum*), pea (*Pisum sativum*), and blackgram (*Vigna mungo*), clearly demonstrate that commonly used herbicides can adversely affect root nodulation and biological nitrogen fixation (BNF), particularly during early growth stages. In contrast, hand weeding consistently resulted in the highest nodule number, biomass, and root development, confirming its compatibility with Rhizobium-legume symbiosis. Among herbicides, the effects on nodulation were generally suppressive but varied with the crop and compound. In soybean, Fenoxaprop-ethyl had a relatively moderate impact, while Quizalofop and Imazethapyr caused notable reductions in nodule formation, especially at higher doses, consistent with prior reports suggesting herbicide interference in rhizobial infection or early signaling (Parsa *et al.*, 2013; Angiras *et al.*, 1995). Similarly, in chickpea and pea, pre-emergence herbicides such as fluchloralin and pendimethalin, and post-emergence mixtures including Topramezone, significantly reduced nodulation and rhizobial populations, particularly at early stages when BNF is critical (Eberbach *et al.*, 2018; Kremer & Means, 2009; Mishra & Bhanu, 2006; Singh, 2005). In black gram, all herbicides reduced nodulation compared to hand weeding, though Quizalofop showed a marginal advantage in nodule biomass despite lower nodule numbers. Although some recovery of nodulation was observed at later stages, early inhibition can limit nitrogen availability and ultimately affect yield (Lyu *et al.*, 2019). These observations underscore the need for careful selection and application of herbicides in legume-inclusive systems. Integrating weed management practices that minimize negative impacts on rhizobial symbiosis is essential for maintaining soil fertility and sustaining crop productivity.

5.2. Decomposition of organic matter

Organic matter decomposition is a key soil ecological process that drives nutrient mineralization, enhances soil structure, and supports microbial diversity, essential for sustainable agriculture. While microbial breakdown of residues releases vital nutrients and forms humus, herbicide-based weed management may disrupt decomposer communities, potentially affecting nutrient cycling and long-term soil health.

5.2.1. Influence of Isoproturon on Decomposition of Surface-Exposed Residues Under Zero-Till Conditions

A controlled study under zero-till wheat conditions examined the effect of isoproturon, a commonly used phenylurea herbicide, on the decomposition of surface-retained residues of rice straw, soybean, and water hyacinth (*Eichhornia crassipes*) (DWR AR, 2006) (Fig. 11).

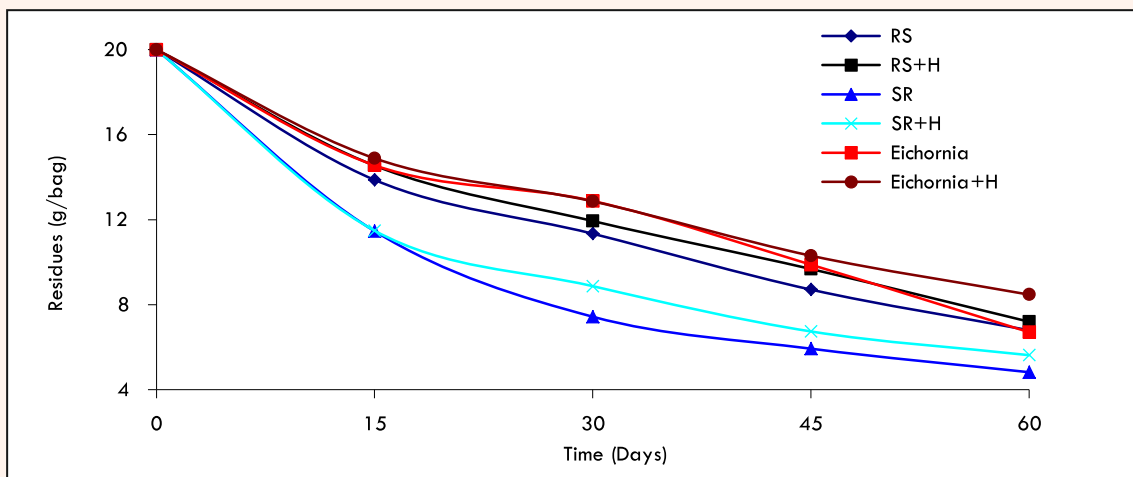


Figure 11: Effect of Isoproturon on decomposition of rice straw, soybean, and water hyacinth in the wheat field (Source: DWR AR 2006)

Across a 60-day period, all untreated residues decomposed progressively, with soybean residues decomposing most rapidly, followed by rice straw and *Eichhornia*. The application of isoproturon (2000 ppm) resulted in a measurable but modest reduction in decomposition across all residue types. Soybean residues treated with isoproturon retained ~1.3 g more biomass than the control, rice straw retained ~0.8 g more, and *Eichhornia* decomposition was reduced by ~1.3 g. These results indicate that isoproturon moderately suppresses microbial decomposition activity, with more pronounced effects on higher-nutrient residues like soybean and *Eichhornia*.

Table 5: Effect of isoproturon on P mineralization during decomposition of plant residues

Herbicide	P content (mg kg ⁻¹)		
	Rice	Soybean	<i>Eichhornia</i>
Control	1.10	1.61	5.91
Isoproturon	1.04	1.72	5.85
Mean	1.07	1.66	5.88
LSD (p = 0.05)	Residue = 0.24		Herbicide = NS

The application of isoproturon had no significant effect (NS) on the phosphorus (P) content of the decomposing plant residues across the three residue types (rice, soybean, and *Eichhornia*) (Table 5). Although minor variations in P content were observed between control and herbicide treatments, these differences were statistically nonsignificant. The mean P content across residues remained stable (2.87 mg kg^{-1}). However, the type of residue significantly influenced P content, as indicated by the LSD value of 0.24. *Eichhornia* showed substantially higher P content compared to rice and soybean residues. These findings suggest that while isoproturon application under zero-till conditions may slightly delay organic matter decomposition, its short-term impact on nutrient release, specifically phosphorus, is negligible. Nevertheless, repeated herbicide exposure could cumulatively affect microbial decomposers and long-term soil function. Prior research has shown that phenylurea herbicides can inhibit soil microbial respiration and enzymatic activity, thereby slowing residue breakdown over time (Zabaloy *et al.*, 2008; Cychón & Piotrowska-Seget, 2015).

5.2.2. Influence of Butachlor on the decomposition of plant residues and microbial activity

A field study employing the nylon mesh bag technique evaluated the impact of butachlor herbicide on the decomposition dynamics and nutrient release from different plant residues, wheat, mustard, chickpea, *Sesbania*, and *Lantana*, under rice cultivation (DWR AR, 2005). Results showed that Butachlor did not significantly affect the decomposition rate or nutrient mineralization of these residues (Varshney & Barman, 2007). Notably, *Lantana* emerged as a cost-effective and nutrient-rich organic source, performing comparably or better than conventional residues like *Sesbania*.

However, butachlor application on black cotton soils adversely affected soil microbial populations and activity. Significant reductions were observed in bacteria, fungi, actinomycetes, *Azotobacter*, and phosphate-solubilizing fungi (PSF), with fungal populations being more sensitive. While total bacterial populations recovered within 30 days at the recommended dose, *Azotobacter*, fungi, and PSF populations remained suppressed even after 45 days. Microbial respiration, indicated by CO_2 evolution, also declined sharply post-application. The addition of glucose at 10 mg/kg soil partially restored microbial activity, suggesting energy supplementation may mitigate herbicide-induced stress (Barman *et al.*, 2009). Butachlor does not hinder plant residue decomposition but significantly suppresses beneficial soil microbial populations and activity, with lasting effects on key functional groups.

5.2.3. Key implications

Herbicide effects on organic matter decomposition in rice–wheat systems are both residue- and herbicide-specific. Isoproturon, applied under zero-tillage, exerted a mild inhibitory effect on the decomposition of surface-exposed residues, with the greatest suppression observed in soybean and *Eichhornia*. However, phosphorus mineralization remained unaffected by herbicide treatment. Field studies under conservation agriculture revealed that Butachlor had no significant effect on the decomposition or nutrient release from various crop residues. Residue type played a more decisive role in influencing nutrient dynamics, with *Eichhornia* exhibiting higher phosphorus content than rice or soybean.

5.3. Major soil enzyme activities

Soil enzymes play a vital role in regulating key biochemical processes that sustain soil health and agricultural productivity. They are primarily produced by soil microorganisms and are essential for nutrient cycling, organic matter decomposition, and maintaining soil fertility. Enzymes such as dehydrogenase, cellulase, and alkaline phosphatase serve as sensitive indicators of microbial activity and soil ecological balance. In the context of modern agriculture, chemical weed management through herbicide application has become widespread; however, its potential to disrupt enzyme-mediated processes raises concerns about long-term impacts on soil ecological functions. The following results represent a consolidated summary of multiple experiments conducted at the Directorate. All herbicides were applied at recommended doses, and soil enzyme activities were measured at the time of harvest.

5.3.1. Dehydrogenase

Soil enzyme activities serve as critical indicators of microbial function and biochemical processes, with dehydrogenase activity in particular representing overall microbial oxidative metabolism and soil respiration, both essential for organic matter decomposition and nutrient cycling (Nannipieri *et al.*, 2017). In given study, the combined herbicide treatment of *Pretilachlor* + *Pyrazosulfuron fb Cyhalofop* + *Penoxsulam* exhibited the most pronounced suppressive effect on dehydrogenase activity, showing a drastic decline of approximately 75% compared to the untreated control (Fig. 12). This significant reduction implies a strong inhibition of soil microbial activity, likely due to the cumulative or synergistic toxicity of multiple active ingredients. Similar findings were reported by Cycoń and Piotrowska-Seget (2015), who observed marked declines in dehydrogenase and other enzyme activities in soils exposed to sulfonylurea and chloroacetamide herbicides. Following this, the treatments *Pendimethalin* + *Imazethapyr fb Topramezone* and *Pendimethalin fb Topramezone* caused dehydrogenase activity reductions of approximately 50%, indicating substantial microbial inhibition. These herbicide combinations, particularly involving HPPD-inhibitors like Topramezone, are known to cause oxidative stress in soil microbial populations (Kalia and Gosal, 2011). The disruption of microbial communities due to such herbicide regimes could account for the observed reduction in metabolic activity. A significant decrease of nearly 35% was also recorded under the *Pretilachlor* + *Pyrazosulfuron fb Bispyribac-sodium (BS) fb Fenoxaprop* treatment. This suggests that repeated herbicide applications in sequential schedules may lead to cumulative stress on microbial populations, reducing their enzymatic capacity over time. Previous research by Zabaloy *et al.* (2012) also supports this notion, where repeated herbicide applications were shown to reduce microbial biomass and enzyme activities in agricultural soils. The combination *Pendimethalin fb Hand Weeding* resulted in a 40% reduction, implying that even with mechanical weed control supplementing chemical application, the initial herbicide exerted notable microbial suppression. Treatments such as *Pretilachlor* + *Pyrazosulfuron fb BS*, *Pretilachlor* + *Pyrazosulfuron fb BS fb Hand Weeding*, and *Clodinafop* + *Metsulfuron fb Hand Weeding* caused moderate reductions ranging from 15% to 25%. These findings suggest that although these treatments were less disruptive compared to more chemically intensive regimes, they still negatively affected microbial respiration to a measurable extent.

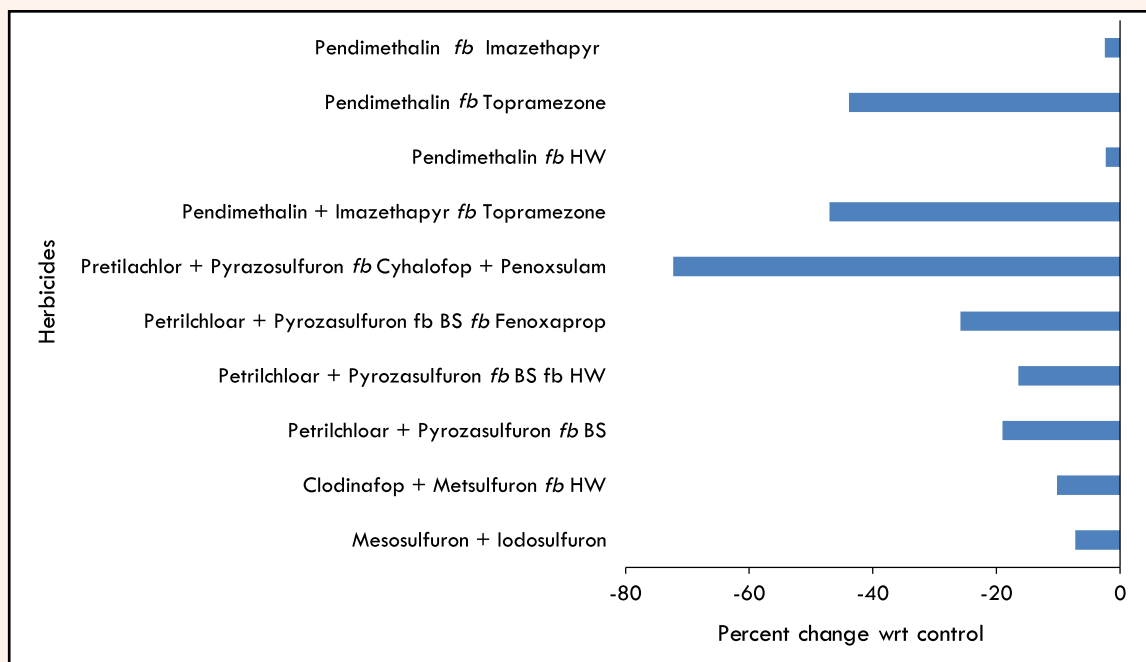


Figure 12: Effect of different herbicides on soil dehydrogenase activity

In contrast, herbicide schedules involving *Pendimethalin* (678 g ha^{-1}) followed by either hand weeding or *Imazethapyr* showed minimal impact on dehydrogenase activity, with reductions ranging between 2% to 5%. This comparatively benign effect may be attributed to the lower persistence and selective toxicity of these compounds, allowing microbial communities to recover or remain unaffected. Supporting this, studies by Tejada (2009) and Rose *et al.* (2016) have shown that herbicides with shorter half-lives or selective action have lesser adverse effects on microbial function and soil health. The least effect was observed in the *Mesosulfuron* + *Iodosulfuron* treatment, which showed only a 1% decline in dehydrogenase activity, suggesting negligible influence on soil microbial activity. Sulfonylurea herbicides, such as mesosulfuron, are typically applied at low doses and are considered less disruptive to soil microbial communities (Gianfreda and Rao, 2004), provided they do not persist in the soil or exhibit synergistic interactions with other agrochemicals.

5.3.2. Cellulase

Cellulase enzymes play a critical role in the decomposition of plant residues by catalyzing the hydrolysis of cellulose into simpler sugars, thereby facilitating carbon cycling and organic matter turnover in soil ecosystems (Nannipieri *et al.*, 2017; Burns *et al.*, 2013). The activity of cellulase enzymes is largely driven by soil microbial populations, particularly fungi and actinobacteria, which are sensitive to agrochemical disturbances. Herbicide application, especially under continuous or high-dosage regimes, can adversely affect cellulase-producing microbes and, consequently, reduce the enzymatic capacity for cellulose degradation (Wang *et al.*, 2024).

In the present study, treatments involving *Pendimethalin fb Imazethapyr*, and *Pendimethalin fb Topramezone* resulted in the most significant suppression of cellulase activity, with reductions nearing 60% compared to the untreated control (Fig. 13). This marked inhibition suggests that Pendimethalin-based herbicide schedules substantially interfere with microbial-mediated cellulose decomposition. Similar findings have been reported by Zabaloy et al. (2008), who noted that herbicide residues, particularly dinitroanilines like Pendimethalin, disrupt fungal biomass and reduce cellulolytic enzyme activities in agroecosystems. However, when *Pendimethalin fb Hand Weeding*, the inhibitory effect on cellulase activity was less. Moreover, the combination of *Pendimethalin + Imazethapyr fb Topramezone* induced a 35% decline in cellulase activity, suggesting that sequential herbicide applications may have additive or synergistic effects on soil biological functions. These observations align with earlier studies demonstrating that herbicides such as Imazethapyr and HPPD-inhibitors like Topramezone can indirectly affect enzyme production by suppressing microbial growth and altering community structure (Kalia & Gosal, 2011; Sannino & Gianfreda, 2001).

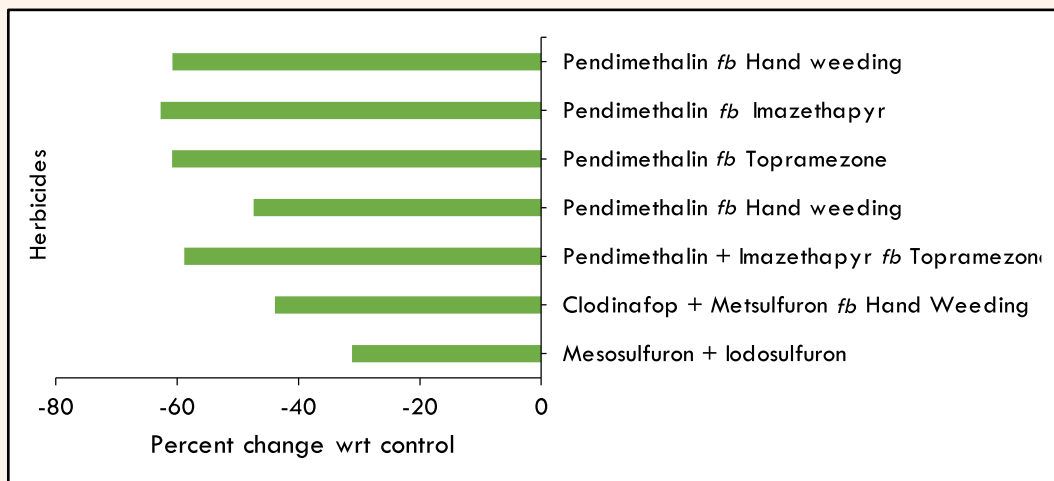


Figure 13: Effect of different herbicides on Cellulase activity

Moderate reductions in cellulase activity, 25% under *Clodinafop + Metsulfuron fb Hand Weeding* and 30% under *Mesosulfuron + Iodosulfuron*, suggest relatively lower disruption to cellulolytic microbial populations compared to more intensive herbicide regimes. This milder impact may be due to the selective mode of action, lower application rates, and rapid degradation of sulfonylurea herbicides like Metsulfuron and Mesosulfuron in soil environments. However, their influence on microbial processes can still vary with soil type and environmental conditions (Cycoñ et al., 2006; Gianfreda & Rao, 2008; Rose et al., 2016).

5.3.3. Alkaline Phosphatase

Soil alkaline phosphatase (ALP) is a crucial extracellular enzyme involved in the mineralization of organic phosphorus compounds, making inorganic phosphate available for plant

uptake. As its activity is closely linked to microbial biomass and root exudation, alkaline phosphatase serves as a sensitive indicator of soil biological health and phosphorus cycling potential (Nannipieri *et al.*, 2011; Acosta-Martínez & Tabatabai, 2000). Herbicide application, depending on its chemical nature and persistence, can influence ALP activity by directly affecting microbial communities or altering the physicochemical environment of the soil.

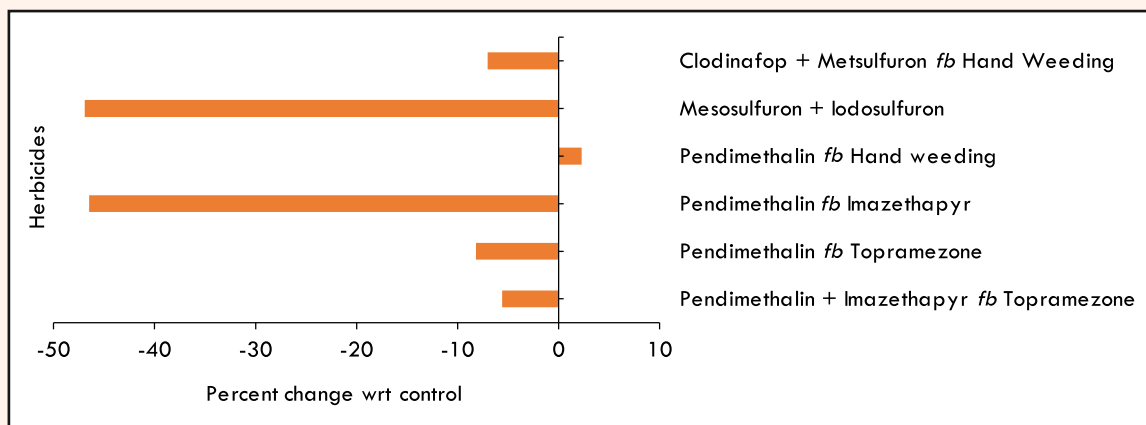


Figure 14: Effect of different herbicides on alkaline phosphatase activity

In the current study, *Mesosulfuron + Iodosulfuron* and *Pendimethalin (PE) fb Imazethapyr* treatments caused the most significant reduction in ALP activity, with a decline of approximately 47% (Fig. 14). This pronounced inhibition suggests a strong suppression of microbial and plant-associated phosphatase-producing systems. Pendimethalin, a dinitroaniline herbicide, has been reported to persist in soil and negatively affect enzymatic activity and microbial diversity (Zabaloy *et al.*, 2008; Cycoń *et al.*, 2010). The addition of Imazethapyr, an ALS-inhibiting herbicide, may further contribute to microbial stress, compounding the adverse effects on phosphorus mineralization processes. Similarly, *Mesosulfuron + Iodosulfuron* resulted in a significant 47% reduction in ALP activity, indicating that even post-emergence sulfonylurea-based treatments can markedly impair soil biochemical functions. These compounds, although applied at low doses, can affect microbial metabolic pathways and enzyme production, particularly when applied repeatedly or in sensitive soils (Gianfreda & Rao, 2008; Wang *et al.*, 2024).

In contrast, moderate reductions were observed under *Pendimethalin fb Topramezone* (10%) and *Clodinafop + Metsulfuron fb Hand Weeding* (8%), suggesting a lesser but still appreciable influence on alkaline phosphatase activity. Topramezone, a 4-HPPD inhibitor, and Metsulfuron, a sulfonylurea, may exert moderate microbial stress, though the extent of impact often depends on soil conditions, application timing, and the presence of mitigating practices such as hand weeding (Sannino & Gianfreda, 2001). Interestingly, the treatments *Pendimethalin + Imazethapyr fb Topramezone* and *Pendimethalin fb Hand Weeding* exhibited minimal reductions in ALP activity, both under 5%. This suggests a potential buffering effect due to either lower cumulative herbicide load or

interactive mitigation from manual weed control, allowing microbial communities to maintain phosphatase expression and function. Such findings are consistent with those of Rose *et al.* (2016), who reported that integrated weed management approaches combining reduced chemical inputs with mechanical methods preserved soil enzyme activities and microbial health.

5.3.4. Key Implications

The study clearly demonstrates that herbicide regimes, particularly those involving multiple active ingredients and sequential applications, significantly suppress critical soil enzyme activities, namely dehydrogenase, cellulase, and alkaline phosphatase, indicating disruption of essential soil functions such as microbial respiration, organic matter decomposition, and phosphorus cycling. Treatments such as Pretilachlor + Pyrazosulfuron *fb* Cyhalofop + Penoxsulam and Pendimethalin-based combinations caused the most severe enzymatic inhibition, reflecting high microbial stress and potential long-term degradation of soil health. In contrast, herbicide programs involving fewer active ingredients or selective, low-dose compounds, such as Mesosulfuron + Iodosulfuron and Pendimethalin *fb* Hand Weeding, exhibited minimal to moderate effects, suggesting a lower ecological footprint and better compatibility with soil microbial functions. These differential impacts emphasize the importance of herbicide selection and application strategy in maintaining soil enzymatic activity. Collectively, the findings highlight that intensive herbicide use can compromise soil biochemical processes critical to agroecosystem sustainability. Therefore, adopting integrated weed management practices that combine chemical and non-chemical methods is crucial to reduce adverse impacts on soil enzyme activity and preserve long-term soil fertility and functionality.

06

Impact of Continuous Herbicide Application on Soil Microbial Population in a Long-Term Rice-Wheat Cropping System

6.1. Effect of continuous use of pre-emergence (rice) and post-emergence (wheat) herbicides on soil microflora

A two-year field study conducted during 2008–2010 at DWR AR investigated the effects of season-specific herbicide regimes on soil microbial populations in rice (*Kharif*) and wheat (*Rabi*), revealing important patterns in microbial responses to chemical weed control strategies (DWR AR, 2010). The aim was to evaluate the effects of various herbicide treatments on soil microbial populations, including bacteria, fungi, and actinomycetes, in comparison with hand weeding and weedy check (untreated control). Soil microbial populations were evaluated at the harvest stage following two years of continuous treatment.

6.1.1. Kharif, Rice

In *Kharif* rice, four weed management treatments were used: Butachlor (1.5 kg ha^{-1}), Anilophos (0.4 kg ha^{-1}), one hand weeding, and a weedy check (control). Since soil sampling was conducted only at the harvest stage, the results represent the net effect of herbicide application over the entire crop growth period (Table 6). At harvest, the application of pre-emergence herbicides, namely butachlor and anilophos, was associated with comparatively lower microbial populations than non-chemical treatments, though some degree of microbial activity was retained, indicating partial tolerance or adaptation of soil microflora to herbicide exposure. Among herbicide treatments, butachlor resulted in the highest bacterial ($2.40 \times 10^6 \text{ cfu g}^{-1}$) and fungal ($1.31 \times 10^3 \text{ cfu g}^{-1}$) populations, followed closely by anilophos. Nevertheless, these values remained significantly lower than those observed in hand-weeded (bacteria: $2.95 \times 10^6 \text{ cfu g}^{-1}$; fungi: $1.98 \times 10^3 \text{ cfu g}^{-1}$) and untreated weedy check plots (bacteria: $3.09 \times 10^6 \text{ cfu g}^{-1}$; fungi: $1.99 \times 10^3 \text{ cfu g}^{-1}$). Actinomycetes, considered more resilient to chemical stress due to their robust physiology and ability to degrade complex organic substrates, exhibited relatively minor variations across treatments, though higher counts were still recorded under non-chemical management (Sarathambal *et al.*, 2015).

Table 6: Effect of continuous use of herbicides on soil microflora in rice at harvest under rice–wheat cropping system in *Kharif*

Treatments	Soil microbial population at harvest (CFU g ⁻¹ dry soil)		
	Bacteria ×10 ⁶	Fungi ×10 ³	Actinomycetes ×10 ³
Butachlor	2.40	1.31	2.13
Anilophos	2.35	1.23	1.94
1 Hand weeding	2.95	1.98	2.20
Weedy check	3.09	1.99	2.18
LSD	0.058	0.048	0.055

(Source: DWR AR, 2010)

6.1.2. Rabi, Wheat

In *Rabi* wheat, five weed management treatments were used: Isoproturon (1.0 kg ha^{-1}), Sulfosulfuron (25 g ha^{-1}), Clodinafop (60 g ha^{-1}), followed by 2, 4-D (0.5 kg ha^{-1}), one-hand weeding, and a weedy check (Control) (Table 7). Among them, isoproturon supported the highest bacterial ($2.52 \times 10^6 \text{ cfu g}^{-1}$) and actinomycetes ($1.95 \times 10^3 \text{ cfu g}^{-1}$) counts, while clodinafop was associated with the highest fungal population ($1.70 \times 10^3 \text{ cfu g}^{-1}$). These variations reflect the selective and differential impacts of herbicide modes of action on microbial taxa, as also reported by Das and Debnath (2006), who observed that phenylurea herbicides like isoproturon are less inhibitory to certain microbial groups than sulfonylureas or auxinic herbicides. Nevertheless, chemical treatments were consistently inferior to non-chemical options in maintaining microbial abundance. The hand-weeded and weedy check plots recorded significantly higher microbial counts across all groups, reaffirming that chemical weed management practices, even when moderately selective, tend to reduce microbial diversity and abundance compared to manual or ecological approaches (Tejada *et al.*, 2011).

Table 7: Effect of continuous use of herbicides on soil microflora in wheat at harvest under rice–wheat cropping system in *Rabi*

Treatments	Soil microbial population at harvest (CFU g ⁻¹ dry soil)		
	Bacteria $\times 10^6$	Fungi $\times 10^3$	Actinomycetes $\times 10^3$
Clodinafop	2.51	1.70	1.82
Sulfosulfuron	2.47	1.71	1.91
Isoproturon	2.52	1.68	1.95
Hand Weeding	2.83	2.16	2.06
Weedy check	2.84	2.21	2.12
LSD	0.105	0.090	0.093

(Source: DWR AR, 2010)

6.1.3. Key Implications

This long-term study highlights that continuous herbicide use in the rice–wheat system exerts a suppressive effect on soil microbial communities, particularly fungi, which appeared most sensitive. While herbicides such as butachlor and isoproturon had comparatively less deleterious impacts, they still induced a temporary decline in microbial populations after application. The microbial recovery observed by harvest time does not negate the ecological risks posed by repeated herbicide exposure, especially in terms of shifts in microbial community structure, enzymatic activity, and overall soil functionality (Nakatani *et al.*, 2014). The consistent superiority of hand weeding and untreated plots in sustaining soil microbial health points to the importance of integrating non-chemical weed control measures in conservation-oriented agriculture. These findings support the growing consensus that sustainable weed management must balance agronomic efficacy with ecological integrity to preserve long-term soil fertility and resilience.

6.2. Effect of continuous use of Bispyribac sodium on soil microbial activity in Kharif rice, in rice–wheat–greengram cropping system in conservation agriculture

The sustainability of herbicide-based weed management practices in conservation agriculture (CA) systems hinges on their compatibility with soil biological processes. In this context,

a two-year field study (2012–2014) was conducted within a rice–wheat–greengram rotation to evaluate the effect of continuous bispyribac sodium use on soil microbial activity under diverse tillage, residue management, and weed control strategies during the Kharif season of 2013–14 (DWR AR, 2014). The study integrated both structural (microbial population) and functional (enzyme activity, soil respiration, nutrient availability) indicators to assess the ecological sustainability of weed management in CA-based rice systems.

A field experiment was conducted over two consecutive cropping seasons (2012–2013 to 2013–2014) to evaluate the long-term effects of continuous herbicide use on soil microbial activity within a rice–wheat cropping system. Specifically, the study aimed to assess the impact of different tillage practices, crop residue management, and weed control strategies on soil microbial populations and functional activity during the *Kharif* season of 2013–14 under varied crop establishment systems (DWR AR, 2014). The main plot treatments included five tillage and crop establishment systems: (1) conventional tillage (CT) with direct-seeded rice (DSR) followed by CT wheat and zero-till (ZT) greengram; (2) CT (DSR) with crop residue (S + R) retention, followed by CT wheat with residue and ZT greengram with residue; (3) ZT (DSR) with crop residue (S) followed by ZT wheat and ZT greengram; (4) ZT (DSR) with full residue retention (S + R) followed by ZT wheat + R and ZT greengram + R; and (5) conventional transplanted rice (CT-TPR) followed by CT wheat. The sub-plot treatments involved three weed management strategies: (1) continuous application of bispyribac sodium with pre-sowing non-selective herbicides, (2) rotational use of herbicides along with pre-sowing non-selective herbicides, and (3) an unweeded control (weedy check). To quantify the impact of these treatments, both basic and functional microbial populations, as well as soil enzyme activities, were assessed at the end of the cropping season.

Continuous application of bispyribac sodium (25 g ha^{-1}) led to a modest increase in total bacterial populations ($8.02 \text{ log cfu g}^{-1}$), exceeding those observed under both the unweeded control ($7.9 \text{ log cfu g}^{-1}$) and rotational herbicide application ($7.6 \text{ log cfu g}^{-1}$) (Table 8).. This suggests that prolonged bispyribac sodium exposure may select for herbicide-tolerant bacterial taxa capable of maintaining population density under chemical stress. Similar findings have been reported by Cycoń *et al.* (2013), who demonstrated microbial adaptation and even proliferation under repeated herbicide applications, attributed to selective enrichment of resistant genera. Fungal populations remained relatively consistent across weed management treatments ($4.1\text{--}4.3 \text{ log cfu g}^{-1}$), highlighting their apparent insensitivity to bispyribac sodium and other herbicide regimes. These results are congruent with prior studies indicating that fungal communities are often less responsive to certain post-emergence herbicides, particularly when soil organic matter and moisture conditions remain favorable (Lo, 2010).

Interestingly, rotational herbicide use supported higher counts of actinobacteria ($5.2 \text{ log cfu g}^{-1}$) and phosphate-solubilizing bacteria ($5.1 \text{ log cfu g}^{-1}$), indicating that diversified herbicide regimes might reduce selection pressure, thereby fostering a more functionally diverse microbial community. Previous studies have emphasized that rotation of herbicides can mitigate the buildup of resistant microbial strains and promote ecological balance (Zhang *et al.*, 2018). Diazotrophic and nitrifying bacterial populations (nitrite oxidizers) were generally stable across treatments,

suggesting a degree of resilience among nitrogen-cycling microbes. This aligns with reports by Johnsen *et al.* (2001), which documented minimal herbicide-induced disruption to nitrogen-transforming microbial populations in soil (Table 8).

Table 8. Effect of continuous use of bispyribac sodium on the population of total and functional microbial groups in rice under DSR-wheat-green gram

Treatments	Basic and functional microbial groups (log cfu g ⁻¹ dry weight of soil)					
	Bacteria	fungi	Actinomycetes	Diazotrophs	Nitrite oxidizers	P-solubilizers
Weedy check	7.9	4.1	4.8	6.7	5.8	4.8
Bispyribac sodium 25g ha ⁻¹	8.0	4.1	4.9	6.6	5.9	4.9
Non-continuous use of herbicide	7.6	4.3	5.2	6.6	5.9	5.1
SEd	0.24	0.13	0.16	0.21	0.18	0.16
LSD (P=0.05)	0.76	0.38	0.47	0.63	0.54	0.49

(Source: DWR AR, 2014)

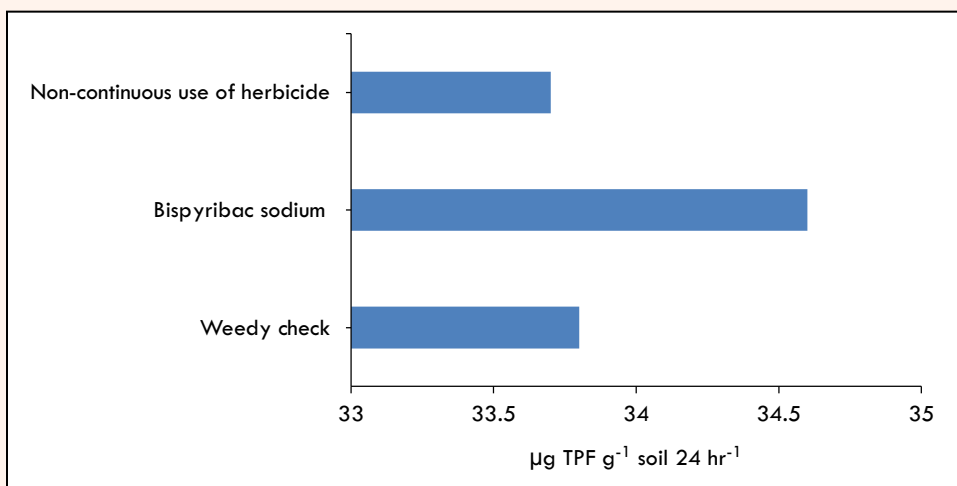


Figure 15: Effect of continuous use of Bispyribac sodium on dehydrogenase activity in soil (Source: DWR AR, 2014)

Dehydrogenase activity, a proxy for overall microbial metabolic activity, was highest under continuous bispyribac sodium application (34.7 µg TPF g⁻¹ soil 24 hr⁻¹), compared to the weedy check (33.8 µg TPF g⁻¹ soil 24 hr⁻¹) and rotational herbicide application (33.6 µg TPF g⁻¹ soil 24 hr⁻¹) (Fig. 15). While this difference is modest, it suggests that sustained herbicide exposure may selectively enhance the activity of microbial communities capable of metabolizing xenobiotic compounds, a phenomenon documented in soils with repeated pesticide use (Wang *et al.*, 2020). However, enhanced enzyme activity under bispyribac sodium treatment does not necessarily equate to greater microbial diversity or ecosystem health, as functional redundancy could mask compositional shifts.

Table 9. Effect of continuous use of herbicides along with tillage practices on soil microbial activity in rice under DSR-wheat-green gram

Treatments	Basic and functional microbial groups (log cfu g ⁻¹ dry weight of soil)					
	Bacteria	fungi	Actinomycetes	Diazotrophs	Nitrite oxidizers	P-solubilizers
<i>Tillage and crop establishment</i>						
DSR-CT+S-CT-ZT	7.7	3.5	4.4	6.6	5.4	5.8
DSR-CT-R+S-CT+R-ZT+R	7.5	3.9	4.7	6.8	5.8	4.3
DSR-ZT+S-ZT-ZT	7.7	4.5	4.9	6.7	6.4	5.3
DSR-ZT+R+S-ZT-ZT	8.5	5.0	5.5	6.9	6.3	4.9
Transplanted rice-CT	7.8	3.0	4.5	6.1	5.4	4.3
SEd	0.34	0.19	0.21	0.30	0.27	0.23
LSD(P=0.05)	1.10	0.61	0.68	0.96	0.87	0.74

(Source: DWR AR, 2014)

Soil microbial communities were significantly shaped by tillage and residue management practices. The zero-tillage direct-seeded rice (DSR-ZT) system with both surface (S) and full residue retention (R) consistently supported higher populations of bacteria (8.5 log cfu g⁻¹), fungi (5.0 log cfu g⁻¹), actinobacteria (5.5 log cfu g⁻¹), and diazotrophs (6.9 log cfu g⁻¹), indicating strong positive effects of residue-mediated organic inputs and reduced soil disturbance (Table 9). These observations align with findings by Sharma et al. (2018), who reported increased microbial biomass and activity under CA practices due to improved soil organic carbon and microclimate conditions. Nitrite oxidizer populations were highest in the DSR-ZT+S system (6.4 log cfu g⁻¹), closely followed by DSR-ZT+R+S (6.3 log cfu g⁻¹), suggesting that surface residue can enhance substrate availability for nitrifying bacteria. Meanwhile, PSB populations peaked under DSR-CT+S (5.8 log cfu g⁻¹), indicating that partial disturbance through conventional tillage combined with surface residue may improve phosphorus mobilization, possibly by altering root-microbe interactions and nutrient cycling dynamics (Yadav *et al.*, 2019). Dehydrogenase activity followed a similar pattern, with DSR-ZT+S showing the highest activity (14.1% higher than CT) (Fig. 17). This was supported by elevated enzyme activity in other CA systems involving either partial or full residue retention, underscoring the benefits of reduced tillage on microbial function. In contrast, conventional puddled transplanted rice (CT-TPR) consistently exhibited the lowest microbial and enzymatic activity, likely due to disrupted microbial habitats, reduced aeration, and physical disturbance.

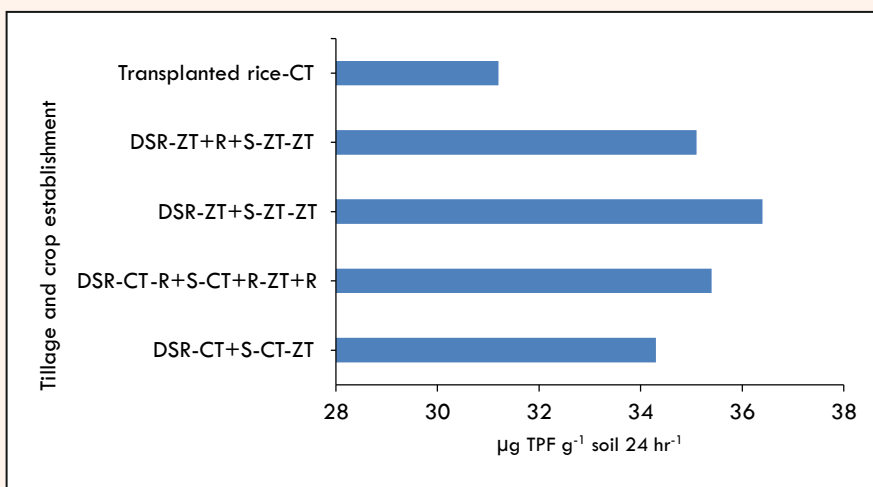


Figure 16: Effect of tillage practices on soil dehydrogenase activity under DSR-wheat-green gram
(Source: DWR AR, 2014)

Among all the treatments, DSR-ZT+S-ZT-ZT exhibited significantly higher activity (14.1%) compared to the control (CT), indicating enhanced microbial activity under continuous zero tillage with surface residue retention (Fig. 16). This was closely followed by DSR-CT-R+S-CT+R-ZT+R and DSR-CT+S-CT-ZT, both of which also exhibited high enzyme activity levels, suggesting that conservation practices involving partial or rotational tillage with residue management positively influence microbial metabolism. In contrast, transplanted rice under conventional tillage (CT) recorded the lowest dehydrogenase activity (just above 30 $\mu\text{g TPF g}^{-1} \text{ soil 24 hr}^{-1}$), highlighting the relatively lower microbial functioning in intensively tilled and puddled systems. The treatment DSR-ZT+R+S-ZT-ZT also showed moderate enzyme activity, further supporting the trend that zero tillage combined with surface residue enhances soil biological health.

Table 10: Effect of continuous use of herbicides along with tillage practices on soil health parameters under DSR-wheat-green gram system

Treatments	Soil respiration (mg CO ₂ h ⁻¹)	SOC (%)	Available N (kg ha ⁻¹)
DSR-CT+S-CT-ZT	5.04	0.63	156
DSR-CT-R+S-CT+R-ZT+R	5.57	0.65	164
DSR-ZT+S-ZT-ZT	4.06	0.67	164
DSR-ZT+R+S-ZT+R-ZT+R	5.06	0.72	163
CT (TPR) -CT	4.42	0.53	130
LSD (P=0.05)	0.87	0.10	21.7

(Source: DWR AR, 2014)

Soil respiration, an integrative measure of microbial activity and organic matter decomposition, was significantly enhanced under conservation treatments. The DSR-CT-R+S-CT+R-ZT+R system recorded the highest rate ($5.57 \text{ mg CO}_2 \text{ h}^{-1}$), reflecting a 26% increase over conventional tillage ($4.42 \text{ mg CO}_2 \text{ h}^{-1}$) (Table 10). Similar improvements were observed in soil organic carbon (SOC), with the DSR-ZT+R+S system achieving a 35.8% increase in SOC over CT (0.72% vs. 0.53%). Available nitrogen was also highest under DSR-CT-R+S and DSR-ZT+S systems (164.6 kg ha^{-1}), a 25.9% improvement compared to CT (130.7 kg ha^{-1}). These findings support earlier research indicating that CA practices enhance microbial-mediated nutrient cycling, carbon sequestration, and overall soil quality (Bhattacharyya *et al.*, 2018; Mangalassery *et al.*, 2015).

6.2.1. Key Implications

Continuous application of bispyribac sodium slightly enhanced total bacterial populations and dehydrogenase activity, indicating a selective stimulation of tolerant microbial communities. However, rotational use of herbicides promoted higher populations of actinobacteria and phosphate-solubilizing bacteria, suggesting that non-continuous herbicide application creates a more favorable environment for functionally beneficial microbes. Fungal populations remained relatively stable across weed management practices, while nitrogen-cycling microbes (diazotrophs and nitrite oxidizers) exhibited resilience to herbicide regimes. Overall, although continuous bispyribac sodium use did not drastically suppress soil microbial activity, rotational herbicide application proved more supportive of microbial diversity and functional groups important for soil health.

6.3. Influence of different herbicides and weed management practices on soil microbial activities in rice and wheat under rice-wheat-green gram cropping system

Based on previous years' studies, a field experiment was conducted during the 2021-2022 to 2022-2023 cropping seasons (*Kharif and Rabi*) to assess in detail the long-term (2 years) impact of continuous herbicide use on soil microbial activity in a rice-wheat cropping system under conservation agriculture (DWR AR, 2021, 2022, 2023). The experiment followed a split-plot design with crop establishment methods as the main plot and weed management practices as the subplot. The main plot consisted of four treatments: T1 – Conventional tillage (CT) for rice, wheat, and green gram; T2 – Zero tillage (ZT) with greengram residue (GR) for rice, ZT with rice residue (RR) for wheat, and ZT with wheat residue (WR) for green gram; T3 – CT for rice, chickpea, and green gram; and T4 – ZT with GR for rice, ZT with RR for chickpea, and ZT with chickpea residue (CR) for green gram. The sub-plot treatments included four weed management strategies.

6.3.1. Kharif, Rice

In rice, weed management treatments were W1: unweeded (control), and W2: application of recommended herbicides (pretilachlor + pyrazosulfuron *fb* bispyribac sodium); W3 represented integrated weed management, which included pre- and post-emergence herbicides supplemented with hand weeding. W4 was herbicide rotation, with different combinations applied over two years, including Pretilachlor + Pyrazosulfuron *fb* Bispyribac Sodium in the first year and Pretilachlor + Pyrazosulfuron *fb* Cyhalofop + Penoxsulam in the second year.

In first year, dehydrogenase activity (DHA), a reliable indicator of overall microbial oxidative activity, was consistently higher in ZT compared to CT, showing a 19.46% increase at 45 days after sowing (DAS) (Fig. 17a). This supports findings by Sharma *et al.* (2016) and Jat *et al.* (2019), who observed improved microbial functioning under conservation tillage due to less soil disturbance and better residue retention.

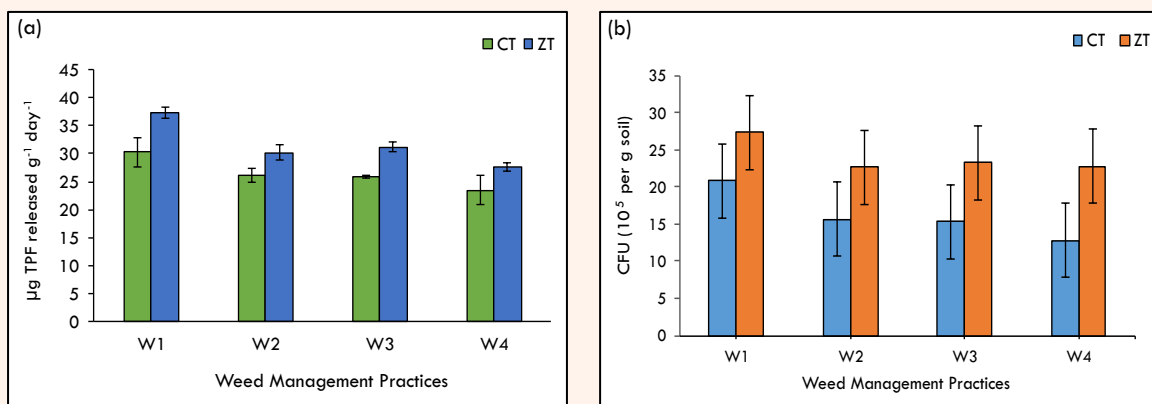


Figure 17: Effect of weed management and tillage practices on (a) Microbial respiration and (b) total culturable bacterial population at 45 days after sowing in rice rhizosphere. Treatments: W1: Unweeded (Control); W2: Pretilachlor + Pyrazosulfuron *fb* Bispyribac Sodium; W3: Pretilachlor + Pyrazosulfuron *fb* Bispyribac Sodium *fb* Hand Weeding; W4: Pretilachlor + Pyrazosulfuron *fb* Bispyribac Sodium (Source: DWR AR, 2021)

Total bacterial populations, as well as cellulose-degrading bacteria (CDB), were markedly enhanced under ZT, with a 48.71% and two-fold increase, respectively, compared to CT (Fig. 17b). These trends suggest that reduced soil disturbance and higher residue inputs in ZT systems may provide more favorable niches and substrates for microbial colonization and activity (Yadav *et al.*, 2021). The positive correlation between CDB and microbial biomass carbon (MBC) ($p < 0.05$) underscores the contribution of cellulolytic microbes to carbon turnover in soil ecosystems (Fig. 18).

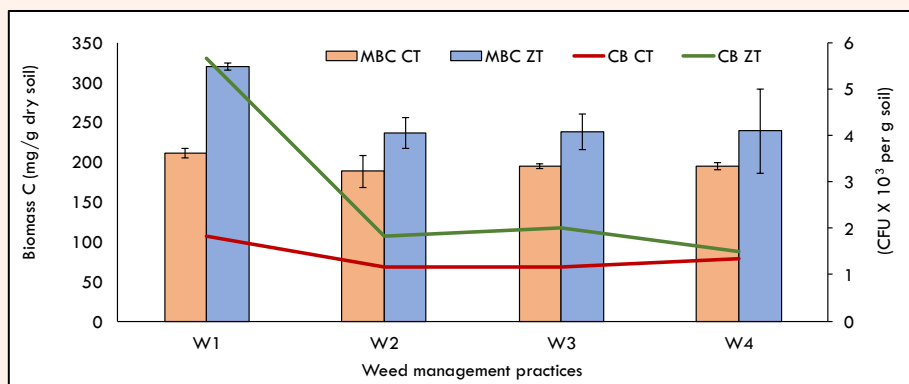


Figure 18: Effect of weed management and tillage practices on soil carbon as evaluated from cellulolytic bacterial population and MBC at 45 days after sowing (Source: DWR AR, 2010)

In the succeeding year, compared to the unweeded treatment (W1), the population of phosphate-solubilizing bacteria (P-solubilizers) was significantly reduced under conventional tillage (CT) by 28%, while no significant difference was observed under the zero-tillage (ZT) system following the application of Cyhalofop + Penoxsulam (W4) (Fig. 19a). These results resonate with findings by Zain *et al.* (2013) and Latha & Gopal (2010), which noted that specific herbicides could differentially affect microbial groups depending on their modes of action and persistence. In the integrated weed management treatment (herbicide + hand weeding) (W3), the microbial population was slightly higher than the control (W1). Dehydrogenase activity was 12% lower in CT compared to ZT.

Additionally, herbicide rotation (W4), especially in the second year with Cyhalofop + Penoxsulam, showed improved microbial respiration and enhanced PSB and fungal populations under ZT (Fig. 19b). This contrasts with continuous application of Bispyribac sodium (W2), which, although maintaining higher MBC and DHA, led to reduced diversity of FLNF, PSB, fungi, and CDB. These observations highlight the potential benefit of rotating herbicide modes of action not only for weed resistance management but also for minimizing ecological disruption in the rhizosphere (Mijangos *et al.*, 2009; Chauhan 2012).

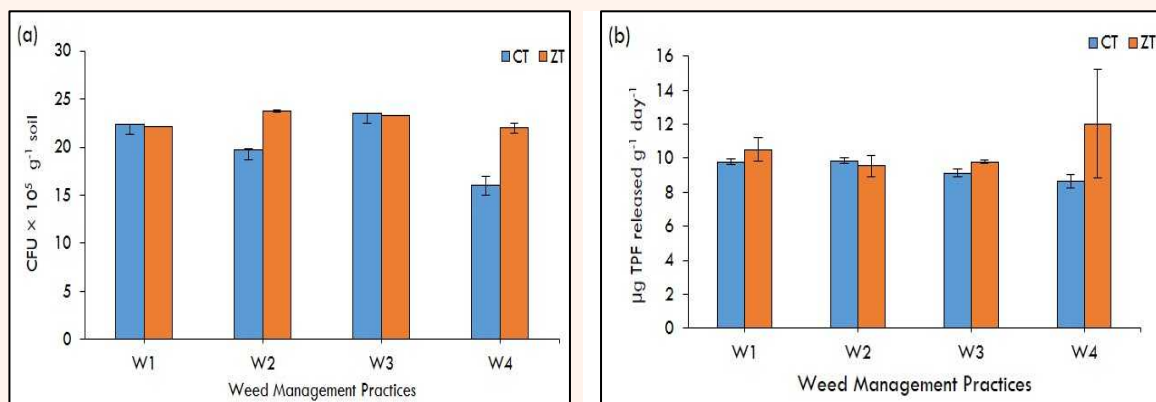


Figure 19: (a) P-solubilizers population, and (d) microbial respiration as influenced by tillage (CT, ZT) and weed management practices (W1, W2, W3, W4) in rice under conservation agriculture. W1: Control; W2: Pretilachlor + Pyrazosulfuron (615 g ha⁻¹) *fb* Bispyribac Sodium (25 g ha⁻¹); W3: Pretilachlor + Pyrazosulfuron (615 g ha⁻¹) *fb* Bispyribac Sodium (25 g ha⁻¹) *fb* hand-weeding; W4: Pretilachlor + Pyrazosulfuron (615 g ha⁻¹) *fb* Cyhalofop + Penoxsulam (135 g ha⁻¹). CFU: Colony Forming Unit (Source: DWR AR, 2022)

6.1.1.1. Multivariate Analysis of Soil Microbial Dynamics in Response to Tillage and Herbicide-Based Weed Management in Rice (Kharif)

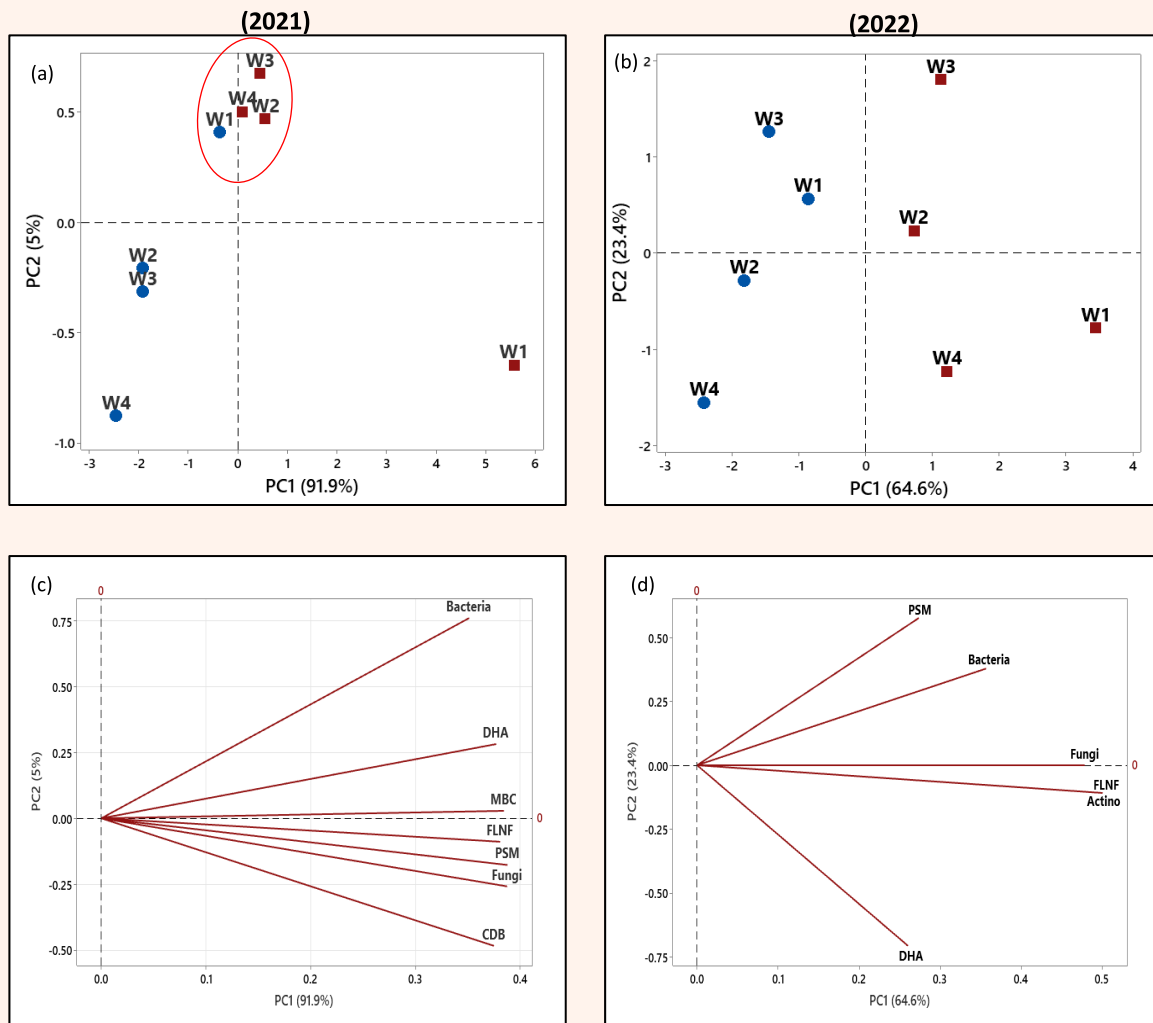


Figure 20: Relationship between tillage and weed management practices and their effect on the microbial population distribution under rice in 2021 [(a), (c)] and 2022 [(b), (d)]. The scores of the first two principal components and (c-d) Loading values. W1: Control; W2: Pretilachlor + Pyrazosulfuron fb BS; W3: Pretilachlor + Pyrazosulfuron fb Bispyribac sodium fb Hand Weeding; W4 (2021): Pretilachlor + Pyrazosulfuron fb BS; W4 (2022): Cyhalofop + Penoxsulam. MBC: Microbial Biomass Carbon, CT (1): Conventional Tillage, ZT (n): Zero Tillage, PSM: P-solubilizing microbes, FLNF: Free-living N-fixers, DHA: Dehydrogenase, CDB: cellulolytic bacteria. (Source: DWR AR, 2023)

The PCA revealed a clear separation of CT and ZT treatments along the first principal component (PC1), indicating distinct microbial community structures influenced by tillage intensity and residue retention. In 2021, PC1 and PC2 explained 91.9% and 16.5% of the total variance, respectively, whereas in 2022, the variance explained was 64.6% and 23.4% (Fig. 20 a&b). This suggests a greater variability in microbial response in the second season, likely due to legacy effects of previous crops and altered weed management strategies. The clustering of ZT treatments (W2, W3, W4) and CT-unweeded control (W1) along PC1 in 2021 suggests a convergence in microbial functional groups under low-disturbance and residue-rich systems. However, this pattern did not persist in 2022, implying that crop residue decomposition and herbicide legacy effects may have caused functional divergence over time. These findings are consistent with earlier reports indicating that ZT systems promote microbial stratification and diversity due to reduced soil disturbance and enhanced organic matter inputs (Helgason *et al.*, 2009; Jat *et al.*, 2019).

In 2021, PC1 was positively associated with microbial biomass carbon (MBC), dehydrogenase activity (DHA), and total bacterial populations. These associations indicate co-occurrence and possibly synergistic enhancement of general microbial activity under certain management practices. However, a negative correlation with free-living nitrogen fixers (FLNF), phosphate-solubilizing bacteria (PSB), fungi, and cellulolytic bacteria (CDB) suggests a potential ecological trade-off or niche separation (Fig. 20c). Similar trade-offs have been observed in herbicide-impacted soils where general microbial respiration increases, but specialized functional groups are suppressed due to altered resource availability or competition (Baćmaga *et al.*, 2015; Cycoń *et al.*, 2013).

Interestingly, this trend was reversed in 2022. PC1 showed positive associations with PSB, total bacteria, and fungi, and negative associations with FLNF, actinomycetes, and DHA (Fig. 20d). This shift indicates a dynamic reorganization of microbial communities possibly driven by changes in herbicide formulation (e.g., cyhalofop + penoxsulam in W4) and seasonal variations in soil moisture, temperature, and residue composition. Such reconfigurations of microbial networks have been documented in long-term rotation trials, where microbial responses evolve over seasons due to legacy effects and interspecies competition (Mijangos *et al.*, 2009; Hartmann *et al.*, 2015). The continuous application of herbicides in W2 (pretilachlor + pyrazosulfuron *fb* bispyribac sodium) was associated with increased MBC, DHA, and total bacterial abundance, indicating that this herbicide regime had a relatively benign or stimulatory effect on general microbial activity. However, the suppression of FLNF, PSM, fungi, and CDB under W2 points toward a selective impact on microbial functional diversity. This aligns with previous findings that sulfonylurea and aryloxyphenoxypropionate herbicides may inhibit sensitive microbial guilds involved in nitrogen fixation and phosphorus solubilization (Lupwayi *et al.*, 2009; Zain *et al.*, 2013).

Integrated weed management (W3), which included herbicide use supplemented with manual weeding, mirrored W2 in microbial trends, suggesting that while hand weeding may support bacterial activity, it may not fully compensate for herbicide-induced suppression of functional groups. Manual weed removal has been shown to improve rhizosphere aeration and

nutrient cycling, but its benefits are often overridden by systemic herbicide residues in the soil matrix (Kremer & Means, 2009).

In 2021, the herbicide rotation treatment (W4) showed microbial dynamics similar to W2, with elevated MBC and bacterial abundance but reduced FLNF and fungal populations. However, in 2022, W4 (cyhalofop + penoxsulam) produced a distinct microbial signature: increased PSB, total bacteria, and fungi, along with reduced DHA and FLNF. This suggests that herbicide rotation may promote microbial diversity in the short term but can also induce shifts in metabolic pathways, potentially suppressing N-fixation and microbial respiration. These findings are consistent with reports that alternating herbicide modes of action can reduce weed pressure while modulating microbial community composition (Chauhan *et al.*, 2012; Topp, 2003).

The PCA-based insights highlight the ecological complexity of soil microbial communities under herbicide and tillage interactions. While ZT and herbicide rotation strategies can enhance general microbial activity and diversity, they may simultaneously suppress functionally important groups like nitrogen fixers. Therefore, the design of weed management programs should balance chemical inputs with practices that support beneficial microbial functions. Adaptive approaches that include crop rotation, residue retention, and moderated herbicide use are essential for sustaining soil health in intensively cultivated agroecosystems (Mandal *et al.*, 2007; Yadav *et al.*, 2021).

6.3.2. Rabi, Wheat

In wheat, weed management treatments were W1: unweeded (control), and W2: application of recommended herbicides (Mesosulfuron + Iodosulfuron); W3 represented integrated weed management, which included Mesosulfuron + Iodosulfuron supplemented with one hand weeding. W4 was herbicide rotation, with different combinations applied over two years, including Clodinafop + Metsulfuron in the first year and Mesosulfuron + Iodosulfuron in the second year.

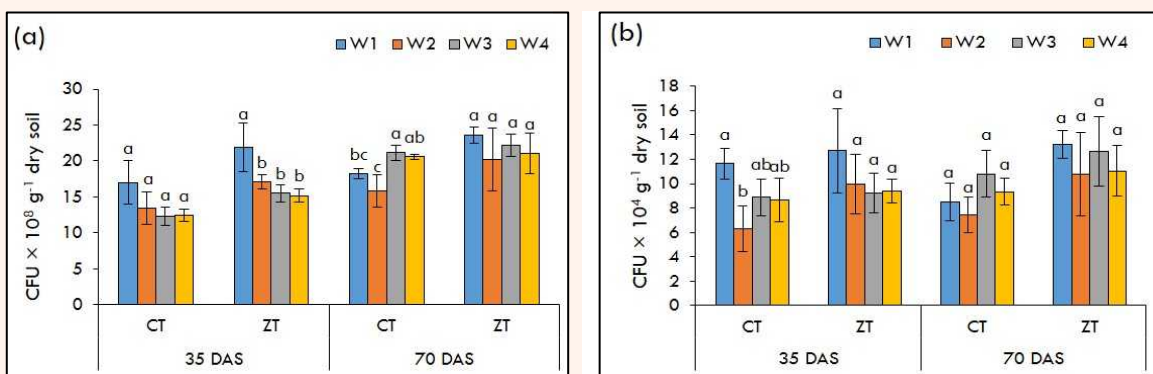


Figure 21: (a) Total bacterial and (b) total fungal population as influenced by tillage (CT, ZT) and weed management practices (W1, W2, W3, W4) in wheat under conservation agriculture. W1: Control; W2: Mesosulfuron + Iodosulfuron; W3: Clodinafop + Metsulfuron fb Hand Weeding; W4: Clodinafop + Metsulfuron (Source: DWR AR, 2021)

In the first year, The application of mesosulfuron + iodosulfuron significantly reduced bacterial populations under conventional tillage (CT), with a 23% decrease at 70 days after sowing (DAS), compared to a relatively milder 16% reduction under zero tillage (ZT) (Fig. 21a). This finding is consistent with earlier studies demonstrating that herbicides belonging to the sulfonylurea group may exert inhibitory effects on bacterial proliferation due to their persistence and interference with microbial enzymatic systems (Cycoń *et al.*, 2013; Lupwayi *et al.*, 2009). In contrast, fungal populations responded positively to ZT, with a consistent 6–28% increase over CT across sampling intervals (Fig. 21b). This enhanced fungal abundance under ZT aligns with previous findings where reduced soil disturbance and higher organic residue levels created a more favorable microhabitat for fungal colonization (Helgason *et al.*, 2009; Jat *et al.*, 2019).

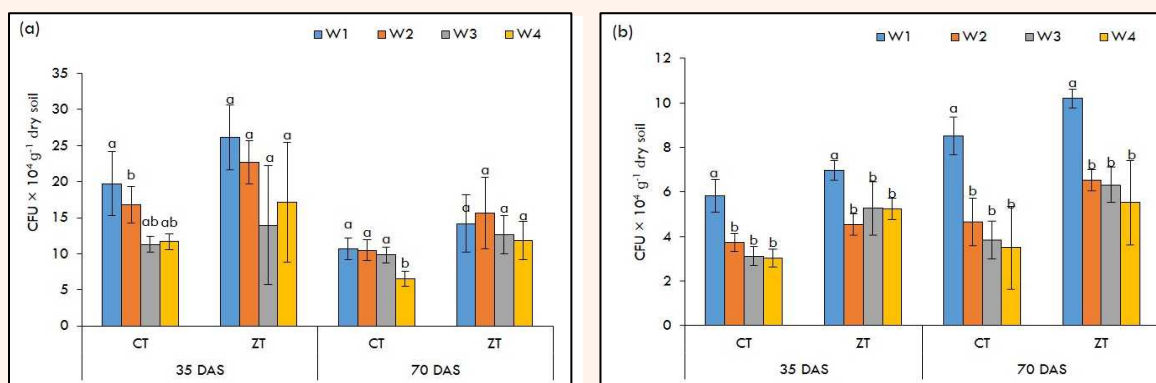


Figure 22: (a) P-solubilizers and (b) free-living N-fixers population as influenced by tillage (CT, ZT) and weed management practices (W1, W2, W3, W4) in wheat under conservation agriculture. W1: Control; W2: Mesosulfuron + Iodosulfuron; W3: Clodinafop + Metsulfuron; W4: Hand Weeding; W4: Clodinafop + Metsulfuron (Source: DWR AR, 2021)

The application of clodinafop + metsulfuron (W4) substantially suppressed phosphate-solubilizing bacteria (PSB) and *Azotobacter* populations under CT at 35 DAS, with a 41–43% and ~59% reduction, respectively (Fig. 22a and b). Mesosulfuron + iodosulfuron also reduced PSB but to a lesser extent (15%). Interestingly, PSB populations recovered under mesosulfuron + iodosulfuron by 70 DAS, indicating a possible adaptive response or degradation of herbicide residues over time. These findings agree with prior studies indicating the transient but significant effect of selective herbicides on soil phosphorus mobilizers and nitrogen fixers (Baćmaga *et al.*, 2015; Latha & Gopal, 2010).

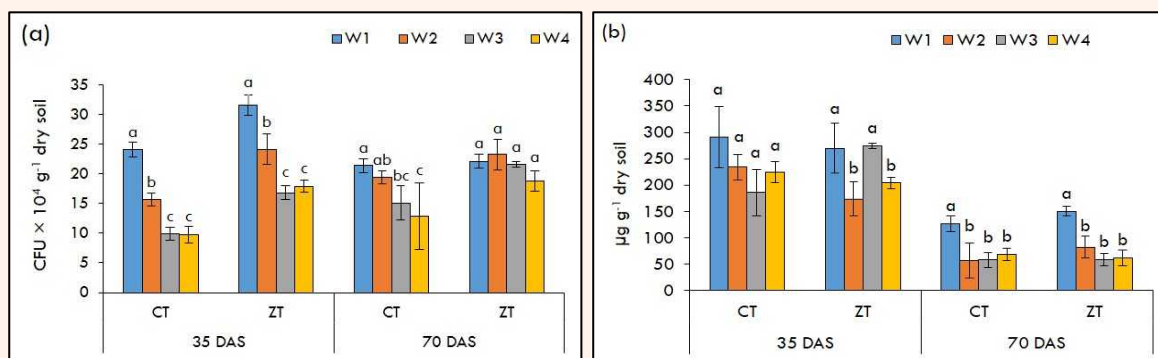


Figure 23: (a) Cellulolytic Bacteria population and (b) Microbial biomass carbon as influenced by tillage (CT, ZT) and weed management practices (W1, W2, W3, W4) in wheat under conservation agriculture. W1: Control; W2: Mesosulfuron + Iodosulfuron; W3: Clodinafop + Metsulfuron *fb* Hand Weeding; W4: Clodinafop + Metsulfuron (Source: DWR AR, 2021)

Cellulolytic bacterial populations showed marked sensitivity to herbicide application, with reductions of 35–59% under CT and 24–46% under ZT at 35 DAS, indicating that both tillage systems are vulnerable to disruption of cellulose-degrading microbial communities (Fig. 23a). These bacteria are integral to organic matter turnover, and their suppression may impact residue decomposition and nutrient cycling in conservation agriculture systems (Mandal *et al.*, 2007; Singh *et al.*, 2020). Microbial biomass carbon (MBC), an integrative indicator of microbial abundance and activity, was unaffected by herbicides under CT at 35 DAS but showed significant suppression under ZT. By 70 DAS, both CT and ZT systems exhibited considerable MBC reductions (55% and 61%, respectively), suggesting cumulative herbicide effects (Fig. 23b). Herbicide-induced reductions in MBC have been widely reported and are attributed to either direct toxicity or reduction in microbial substrates due to inhibited root growth and exudation (Chagnon *et al.*, 2013; Kremer & Means, 2009).

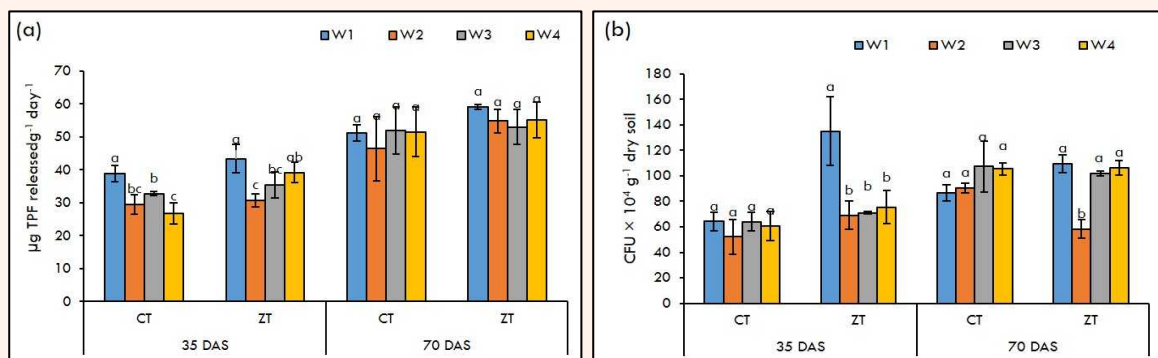


Figure 24: (a) Dehydrogenase and (b) Alkaline phosphatase activity as influenced by tillage (CT, ZT) and weed management practices (W1, W2, W3, W4) in wheat under conservation agriculture. The histogram represents the mean values of three replicates followed by the standard deviation error bars. The same alphabet denotes that the means are significantly not different ($\alpha=0.05$). W1: Control; W2: Mesosulfuron + Iodosulfuron; W3: Clodinafop + Metsulfuron *fb* Hand Weeding; W4: Clodinafop + Metsulfuron (Source: DWR AR, 2021)

Dehydrogenase activity (DHA), a proxy for overall microbial respiration and metabolic activity, was consistently higher under ZT, being 1.2 times greater than CT ($p < 0.05$). This supports the view that minimal disturbance systems enhance microbial respiration due to improved soil structure and moisture retention (Mijangos *et al.*, 2009). However, herbicide application under both systems reduced microbial respiration by 9–31% at 35 DAS, though the decline became statistically non-significant by 70 DAS, indicating transient inhibition (Fig. 24a). Alkaline phosphatase, a key enzyme involved in phosphorus cycling, was significantly suppressed (31–40%) under ZT in mesosulfuron + iodosulfuron treatments, highlighting selective enzymatic inhibition under reduced tillage (Fig. 24b).

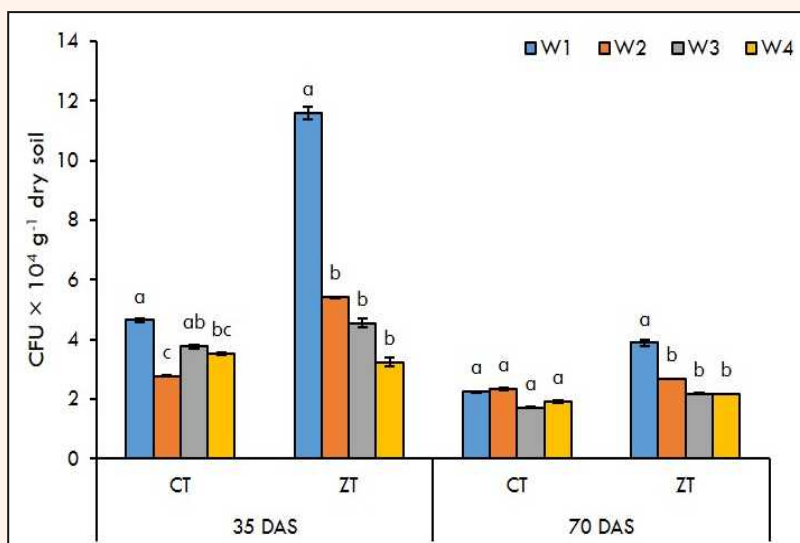


Figure 25: Cellulase enzyme activity as influenced by tillage (CT, ZT) and weed management practices (W1, W2, W3, W4) in wheat under conservation agriculture. W1: Control; W2: Mesosulfuron + Iodosulfuron; W3: Clodinafop + Metsulfuron fb Hand Weeding; W4: Clodinafop + Metsulfuron (Source: DWR AR, 2021)

Similarly, cellulase activity, which plays a role in organic matter breakdown, was significantly decreased by clodinafop + metsulfuron, correlating with the observed MBC decline ($p < 0.05$) (Fig. 25a). These trends are consistent with studies indicating that certain herbicides can selectively inhibit microbial enzymes involved in carbon and phosphorus cycling (Zain *et al.*, 2013; Topp, 2003). Urease activity, critical for nitrogen mineralization, was suppressed by herbicide treatments at 35 DAS under both tillage systems. Notably, under W3 (herbicide + hand weeding), urease activity rebounded significantly at 70 DAS, suggesting that integrating manual weed control may help mitigate some herbicide-induced stress on nitrogen cycling pathways (data not shown).

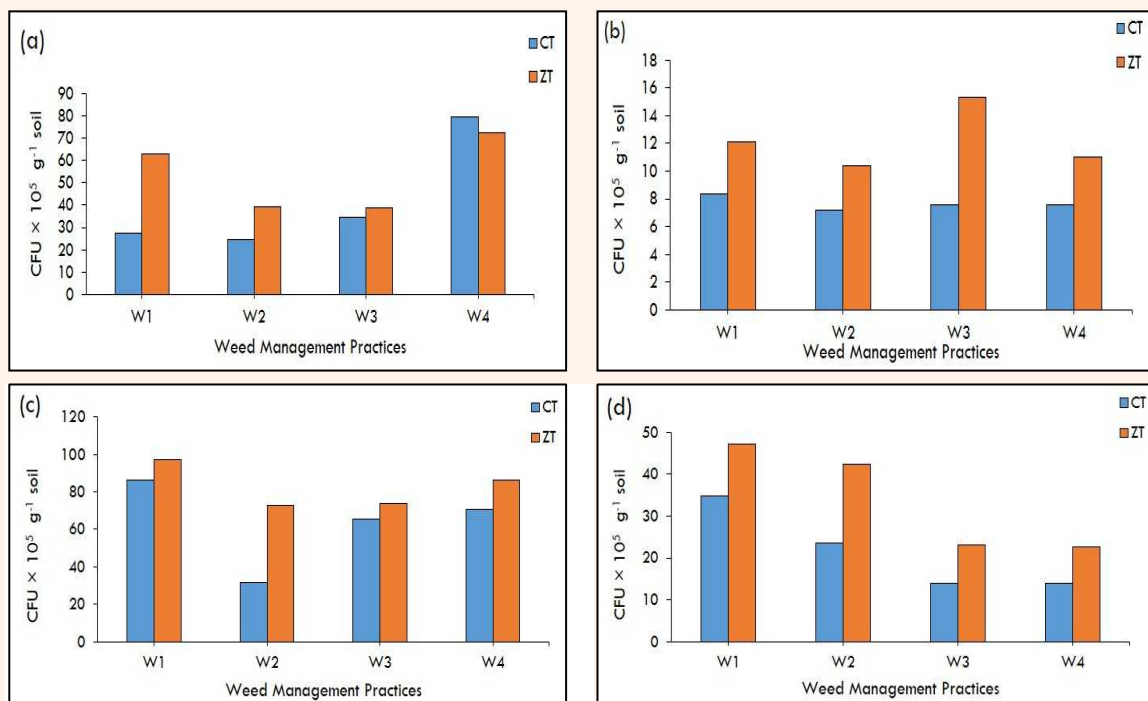


Figure 26: Microbial population (a) total bacteria, (b) fungi, (c) P-solubilizers, and (d) actinomycetes as influenced by tillage (CT, ZT) and weed management practices (W1, W2, W3, W4) in wheat under conservation agriculture. W1: Control; W2: Mesosulfuron + Iodosulfuron (12.2 + 2.4 g ha⁻¹); W3: Clodinafop + Metsulfuron (60 + 4 g ha⁻¹) (POST) *fb* Hand weeding; W4: Mesosulfuron + Iodosulfuron (12.2 + 2.4 g ha⁻¹) (Source: DWR AR, 2022)

In the second year (2022–23), mesosulfuron + iodosulfuron demonstrated a stimulatory effect on PSB and total bacterial populations, reflecting possible microbial adaptation or a decline in herbicide residues (Fig. 26). Conversely, clodinafop + metsulfuron selectively enhanced nitrogen-fixing bacterial populations, suggesting that herbicide combinations exert differential pressure on microbial guilds depending on their chemical properties and soil interactions (Tedersoo *et al.*, 2020; Vischetti *et al.*, 2008).

Phospholipid fatty acid (PLFA) analysis offers a powerful, culture-independent tool for characterizing the structural composition and physiological status of microbial communities in soil ecosystems. In the present study, PLFA profiling was utilized to discern the effects of herbicide-based weed management practices and contrasting tillage systems, conventional tillage (CT) and zero tillage (ZT), on microbial biomass, community structure, and stress physiology in the wheat phase of a rice-wheat-greengram cropping system. Total PLFA content, an indicator of living microbial biomass, was substantially higher under ZT than CT, with ZT exhibiting a 27.5% increase in microbial PLFA across treatments. The highest PLFA content (167 nmol g⁻¹) was observed under clodinafop + metsulfuron (W4) in ZT, compared to 125 nmol g⁻¹ under W3 (clodinafop + metsulfuron *fb* hand weeding) in CT (Fig. 27a). These results align with previous research suggesting that reduced soil disturbance in ZT preserves soil structure and enhances organic matter retention,

thereby providing a favorable habitat and energy substrate for microbial proliferation (Six *et al.*, 2006; Helgason *et al.*, 2009). Additionally, ZT allows for residue retention and rhizosphere enrichment, which collectively stimulate microbial growth and activity (Jat *et al.*, 2019; Mbuthia *et al.*, 2015). The Shannon–Wiener diversity index (H'), a composite measure of microbial richness and evenness, was slightly higher under ZT (1.47) than CT (1.43) (Fig. 27b). Though the difference is modest, it reinforces the conclusion that ZT fosters a more biologically diverse and stable microbial community. Similar marginal gains in microbial diversity under conservation tillage have been documented in long-term field studies and are attributed to improved soil organic carbon, root exudation patterns, and habitat heterogeneity (Lupwayi *et al.*, 2001; Sharma *et al.*, 2021).

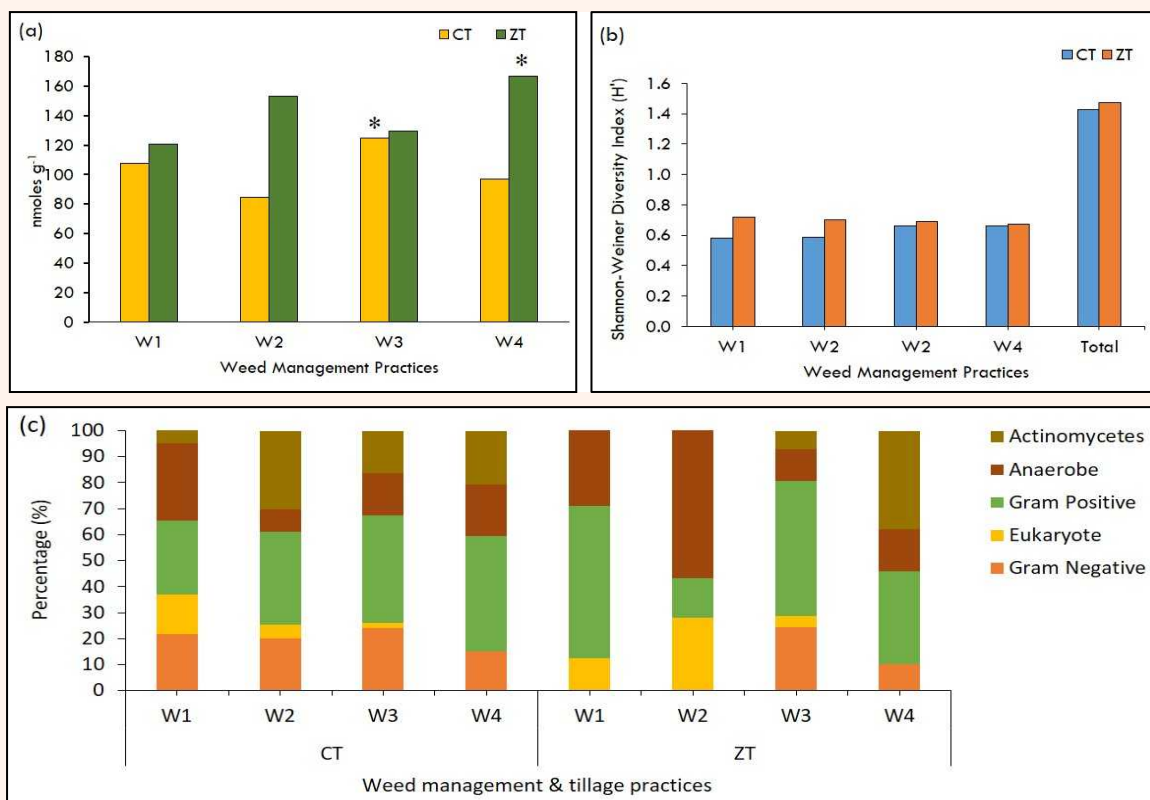


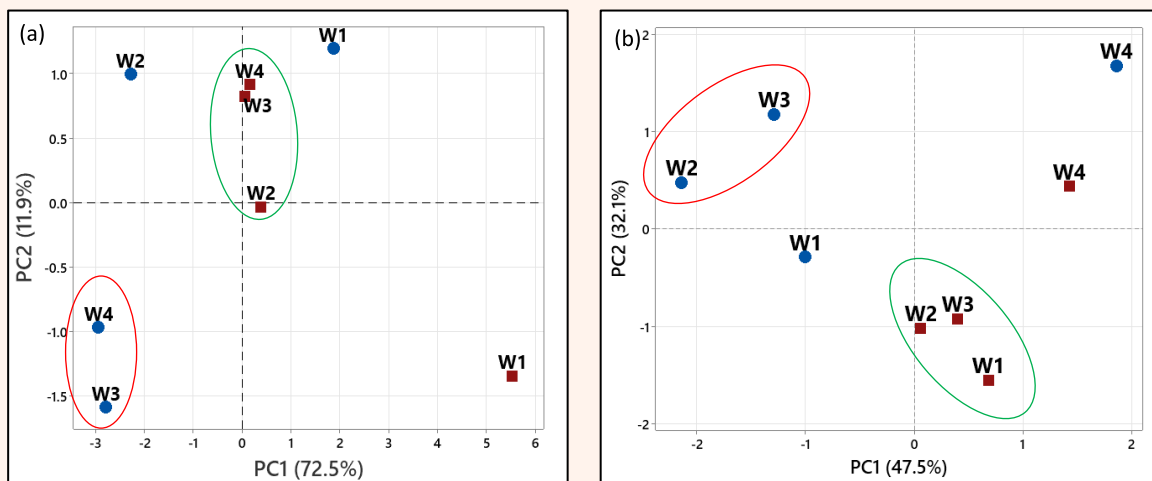
Figure 27: Soil Microbial PLFA: (a) Total PLFA, (b) Shannon-Weiner diversity index and (c) Fatty acid type converted to microbial groups as influenced by tillage (CT, ZT) and weed management practices (W1, W2, W3, W4) in wheat under conservation agriculture. W1: Control; W2: Mesosulfuron + Iodosulfuron; W3: Clodinafop + Metsulfuron *fb* Hand Weeding; W4: Clodinafop + Metsulfuron. CT: Conventioanl tillage, ZT: Zero-tillage (Source: DWR AR, 2023)

The Gram-positive to Gram-negative (G+/G-) PLFA ratio—a widely used indicator of microbial community adaptation to stress, particularly related to nutrient limitation or physical disturbance was significantly modulated by both tillage and herbicide practices. Under CT,

herbicide treatments increased the G+/G- ratio by 38–125%, with the highest values observed under W4. In contrast, under ZT, the ratio decreased by 12–84% relative to the unweeded control (W1), indicating reduced microbial stress in the absence of frequent soil disturbance (Fig. 27c). Gram-positive bacteria, due to their thicker peptidoglycan cell walls, are typically more resistant to environmental stresses and dominate under harsh or resource-limited conditions, while Gram-negative bacteria thrive in nutrient-rich environments (Bossio & Scow, 1995; Kaurin *et al.*, 2022). Therefore, the elevated G+/G- ratio under CT implies a community shift toward more stress-tolerant taxa, likely driven by combined herbicide exposure and mechanical disruption. Conversely, the decline in this ratio under ZT supports the premise that reduced tillage and residue cover ameliorate environmental stresses, enhancing microbial community resilience and functional potential. Anaerobic PLFA biomarkers were 1.5-fold higher under ZT than CT, suggesting the development of localized anaerobic microsites due to surface residue cover and limited soil aeration (Fig. 27c). This observation is consistent with studies that report elevated anaerobic microbial activity in conservation tillage systems, particularly in regions of high residue input and limited oxygen diffusion (Fierer *et al.*, 2003; Franzluebbers *et al.*, 1995). While this may indicate enhanced organic matter turnover under facultative anaerobic pathways, it could also signal potential redox stress if drainage is poor or residue decomposition exceeds oxygen supply.

Moreover, the application of clodinafop + metsulfuron under ZT (W4) resulted in the highest microbial biomass without a corresponding spike in stress indicators (i.e., G+/G- ratio), suggesting that herbicide rotation under reduced tillage may promote a more functionally efficient and resilient microbial community. This supports previous evidence indicating that rotating herbicide modes of action, especially when integrated into conservation agriculture systems, can minimize adverse ecological impacts while maintaining weed control efficacy (Chauhan *et al.*, 2012; Topp, 2003).

6.3.2.1. Multivariate Analysis of Soil Microbial Dynamics in Response to Tillage and Herbicide-Based Weed Management in Wheat (Rabi)



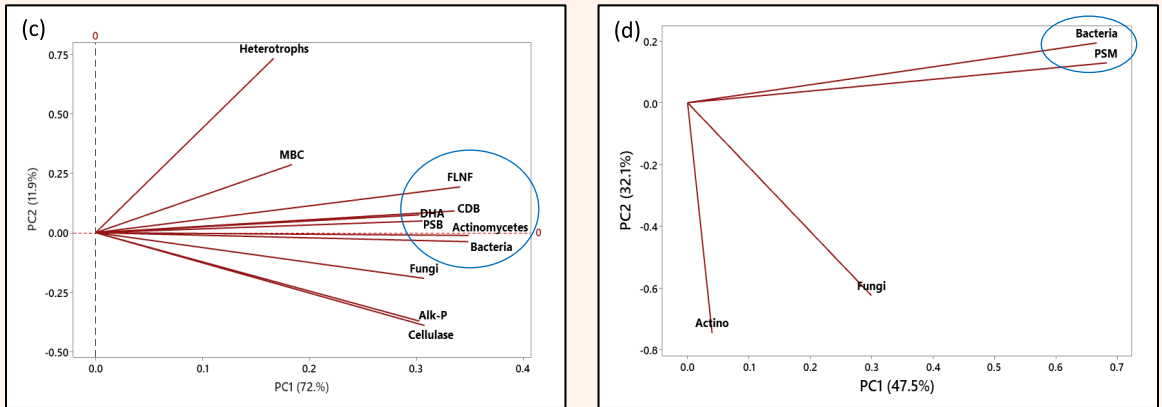


Figure 28: Relationship between tillage and weed management practices and their effect on the microbial population distribution under wheat in 2021-22 [(a), (c)] and 2022-23 [(b), (d)]. The scores of the first two principal components and (c-d) Loading values. W1: Control (unweeded); W2: Mesosulfuron + Iodosulfuron; W3: Clodinafop + Metsulfuron fb Hand Weeding; W4 (2021-22): Clodinafop + Metsulfuron; W4 (2022-23): Mesosulfuron + Iodosulfuron. MBC: Microbial Biomass Carbon, CT (1): Conventional Tillage, ZT (n): Zero Tillage, PSM: P-solubilizing microbes, FLNF: Free-living N-fixers, DHA:

Understanding the impact of agronomic practices on soil microbial dynamics is fundamental for designing sustainable cropping systems. In this study, principal component analysis (PCA) was applied to explore shifts in microbial communities under different tillage regimes and weed management strategies during two consecutive *Rabi* (winter) wheat seasons. Principal component analysis (PCA) was employed to assess the impact of tillage and herbicide-based weed management practices on soil microbial communities over two consecutive *Rabi* seasons. In 2021-22, PC1 and PC2 explained 72.5% and 11.9% of the variance, respectively, whereas in 2022-23, they accounted for 47.5% and 32.1%, indicating a shift in microbial responses across years (Fig. 28a&b). In the 2021-22 season, unweeded ZT plots exhibited the highest microbial abundance, followed by CT-unweeded and herbicide-treated plots under ZT. Herbicide applications under CT (W2-W4) were associated with the lowest microbial populations, suggesting that conventional tillage exacerbates herbicide-induced microbial suppression. This is consistent with previous studies showing that CT disrupts soil structure and reduces microbial activity (Lupwayi et al., 2001; Helgason et al., 2009). In contrast, during the 2022-23 season, mesosulfuron + iodosulfuron (W4) treatments showed higher microbial populations in both CT and ZT, possibly due to microbial adaptation or selective stimulation (Baćmaga et al., 2015; Cycoń et al., 2013). This highlights the role of herbicide legacy and season-specific interactions in shaping microbial responses (Fig. 28c & d).

PCA consistently separated ZT from CT along PC1, underlining tillage as the primary factor influencing microbial structure. Zero tillage favored greater microbial abundance and diversity due to minimal soil disturbance and improved habitat stability (Jat *et al.*, 2019; Sharma *et al.*, 2021). In CT, unweeded plots (W1) were negatively correlated with PC2, while herbicide-treated plots (W2, W3) showed positive correlations, suggesting that weed removal methods distinctly affect microbial communities in disturbed soils. PC1 loading scores indicated year-wise shifts in total bacterial and phosphate-solubilizing bacterial (PSB) populations. In 2021–22, PSB populations were highest under unweeded ZT, while herbicide treatments reduced them under CT. In 2022–23, mesosulfuron + iodosulfuron (W4) significantly increased PSB and bacterial populations in both tillage systems, indicating a potential selective stimulatory effect of this herbicide combination (Singh *et al.*, 2020).

6.3.3. Key implications

6.3.3.1. Microbial Shifts under Herbicide Use

Continuous herbicide application in the rice–wheat–greengram system under conservation agriculture led to herbicide- and management-specific shifts in soil microbial diversity and function. Over two years, herbicide-only treatments (W2) under conventional tillage (CT) significantly reduced microbial respiration, biomass carbon, and the abundance of key functional groups, including free-living nitrogen fixers, phosphate-solubilizing bacteria, fungi, and cellulolytic bacteria, compared to integrated weed management (W3). In rice, pretilachlor + pyrazosulfuron followed by bispyribac sodium maintained total bacterial counts but suppressed nutrient-cycling taxa. In CT wheat, mesosulfuron + iodosulfuron markedly decreased bacterial populations, urease and phosphatase activity, and respiration during early growth stages. Rotational herbicide regimes (W4) initially preserved microbial biomass and respiration but altered community composition, indicating selective microbial enrichment.

6.3.3.2. Tillage Effects

Zero tillage (ZT) consistently supported higher microbial diversity, biomass carbon, and enzyme activity compared to CT. ZT soils maintained more stable and resilient microbial communities, even under herbicide pressure, highlighting its protective role in microbial conservation. In contrast, CT soils experienced pronounced declines in microbial health when subjected to chemical weed management.

6.3.3.3. Comparative Impact of Weed Management Strategies

Integrated weed management (W3), combining herbicides with manual weeding, and weedy check plots maintained significantly higher microbial populations and enzymatic activities across both seasons, causing minimal disruption to microbial habitats. Conversely, continuous herbicide use under CT led to notable suppression of microbial biomass and functional diversity. Rotational herbicide use with diverse modes of action proved more sustainable than continuous single-mode application.

6.3.3.4. *Herbicide-Specific Effects*

Herbicide impact varied by type and crop season. In the kharif season, herbicides like butachlor, anilophos, and bispyribac sodium suppressed microbial populations, particularly fungi, with butachlor showing relatively milder effects. In the rabi season, isoproturon was less harmful to beneficial microbes, while clodinafop + metsulfuron and mesosulfuron + iodosulfuron had strong negative effects, reducing populations of *Azotobacter* and phosphate-solubilizing fungi.

6.3.3.5. *Functional Microbial Indicators of Soil Health*

Microbial biomass carbon, phosphate-solubilizing organisms, dehydrogenase, and cellulolytic bacteria emerged as sensitive indicators of soil health. Changes in these parameters reflected herbicide-induced nutritional stress and microbial imbalance. Their monitoring can provide early warning signals of ecological disturbance in conservation agriculture systems.

07

Conclusion

The impact of chemical weed management practices, specifically the use of herbicides, on agriculturally beneficial microorganisms and their ecological functions in rice-wheat cropping systems is multifaceted and herbicide-specific, ranging from moderate suppression to significant detrimental effects. While these practices are effective for controlling weed infestations, the findings from this comprehensive assessment highlight their potential to influence essential soil microbial communities and disrupt key ecosystem functions critical for sustainable crop production. The extent of these effects varies depending on the type of herbicide, its application rate, and the overall management strategy employed. Herbicides such as clodinafop, isoproturon, 2,4-D, and metribuzin were consistently detrimental to vital microbial groups, including vesicular arbuscular mycorrhizal (VAM) fungi, plant growth-promoting rhizobacteria (PGPR), phosphate-solubilizing fungi (PSF), and biocontrol agents like *Trichoderma*. These organisms are integral to nutrient cycling, plant health, and soil fertility. Although some herbicides, such as sulfosulfuron, fenoxaprop, and butachlor, exhibited relatively lower toxicity, their impact was still more severe than non-chemical methods like hand weeding or integrated weed management (IWM), which consistently preserved microbial abundance and diversity.

The adverse effects of herbicides extend beyond microbial populations to impair core soil functions. Biological nitrogen fixation (BNF), particularly in legumes like soybean, chickpea, pea, and black gram, was significantly hampered by herbicides, which reduced nodulation and rhizobial colonization during early growth stages. Likewise, herbicide treatments suppressed essential soil enzyme activities, including dehydrogenase, cellulase, and alkaline phosphatase, enzymes crucial for microbial respiration, organic matter decomposition, and phosphorus cycling. Multicomponent or sequential herbicide applications caused the greatest disruptions, indicating a strong ecological cost associated with intensive chemical use.

In long-term studies, continuous and sole reliance on herbicides in conservation agriculture systems led to notable declines in microbial biomass, respiration, and functional microbial communities. These effects were more pronounced under conventional tillage, whereas zero-tillage practices offered a buffering effect by sustaining higher microbial activity and diversity. Moreover, rotational herbicide use and IWM strategies were shown to mitigate negative impacts, preserve beneficial microbes, and support enzymatic and nutrient-cycling functions critical to sustainable crop production.

Overall, the ecological sustainability of rice-wheat cropping systems depends heavily on how weed management practices interact with the soil microbiome. To ensure long-term soil health, productivity, and ecological balance, weed management strategies must shift from continuous, broad-spectrum herbicide use to more nuanced approaches. This includes adopting IWM principles that blend selective herbicide use with cultural and mechanical methods, practicing herbicide rotation, and promoting conservation tillage. Incorporating microbial impact assessments into herbicide evaluation protocols is vital for safeguarding the biological integrity of agroecosystems and securing the future of sustainable agriculture.

08

Way Forward

The findings of this comprehensive assessment highlight the necessity of a paradigm change in weed control techniques for rice-wheat cropping systems to protect ecologically important microorganisms and their agricultural benefits. A scientifically informed, ecologically balanced approach must be adopted to mitigate the adverse effects of chemical weed management on soil microbial health and ensure long-term sustainability. The following strategies are recommended:

Adopt Integrated Weed Management (IWM): Integrated weed management, combining chemical, mechanical, manual, and cultural control methods, should be prioritized to reduce herbicide reliance and minimize disturbance to soil microbial communities. This approach allows for more stable and resilient soil ecosystems while maintaining effective weed suppression.

Promote Herbicide Rotation and Diversification: Continuous use of herbicides with the same mode of action under conventional tillage can lead to microbial suppression and reduced functional diversity. Rotational use of herbicides with different biochemical targets can mitigate selection pressure on microbial communities and reduce long-term ecological risks.

Encourage Selective and Site-Specific Herbicide Application: Herbicides with comparatively lower toxicity to beneficial microorganisms, such as isoproturon or sulfosulfuron, should be preferred. Site-specific herbicide recommendations, based on soil type, microbial sensitivity, and cropping history, can help maintain microbial diversity and functionality.

Integrate Microbial Impact Assessments in Herbicide Evaluation: Herbicide risk assessments should routinely include evaluations of their effects on key microbial groups such as vesicular-arbuscular mycorrhizal (VAM) fungi, plant growth-promoting rhizobacteria (PGPR), *Trichoderma* spp., and nitrogen-fixing bacteria. This will ensure that weed control strategies do not inadvertently disrupt essential nutrient-cycling processes and soil fertility.

Promote Conservation Tillage and Residue Retention: Zero tillage and reduced tillage practices, coupled with in-situ crop residue retention, provide a favourable habitat for soil microbes by minimizing physical disturbance and enhancing organic inputs. These practices buffer the negative impacts of herbicides and promote the recovery and resilience of microbial communities.

Preserve Legume-Based Rotations and Symbiotic Associations: Care must be taken to avoid herbicides that adversely affect legume-rhizobia symbiosis, which is critical for biological nitrogen fixation. Where possible, integrate manual weeding or use legume-compatible herbicides in legume-inclusive rotations to maintain soil nitrogen status and reduce fertilizer dependence.

Strengthen Research and Farmer Awareness: Continued research is essential to identify herbicides that are more compatible with soil microbial health and to develop microbiome-friendly weed control strategies. Simultaneously, farmer training programs should promote awareness of the long-term ecological impacts of herbicide overuse and the benefits of integrated weed management practices.

By integrating these strategies, it is possible to balance effective weed control with the conservation of beneficial soil microbial communities, thereby enhancing soil biological health, nutrient cycling, and long-term agricultural productivity in the rice-wheat system.

09

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