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Assessment of herbicide-induced changes in beneficial microflora of Bajra (*Pennisetum glaucum* L.) rhizosphere

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ABSTRACT

The present study investigates the impact of various herbicides on beneficial microbial populations in the rhizosphere of *Pennisetum glaucum* (Bajra), focusing on *Azotobacter*, *Azospirillum*, and phosphate-solubilizing bacteria (PSB). Herbicidal treatments significantly influenced microbial populations at different crop growth stages, with a peak observed at 60 days after sowing (DAS). Among pre-emergence herbicides, Pendimethalin consistently supported the highest populations of all three microbial groups, while Oxyfluorfen exhibited suppressive effects. Phenaxoprop-ethyl emerged as the most favorable post-emergence herbicide, enhancing microbial proliferation, whereas Imazethapyr consistently reduced microbial abundance. Sequential application of Pendimethalin + Propaquizafop-ethyl maintained the highest microbial populations, while the combination with Imazethapyr was the least favorable. Microbial populations declined slightly by harvest, though treatment trends remained consistent. The findings highlight Pendimethalin and Phenaxoprop-ethyl as microbiologically compatible options, while Imazethapyr poses potential risks to rhizospheric health. These results emphasize the importance of selecting herbicides not only for weed management efficacy but also for their compatibility with beneficial soil microflora crucial for nutrient cycling and plant productivity. The study underscores the ecological benefits of integrating herbicide strategies with microbial sustainability, recommending Pendimethalin + Propaquizafop-ethyl for promoting rhizospheric microbial health in sustainable bajra cultivation.

KEY WORDS: *Herbicides*; *Rhizosphere microflora*; *Bajra*; *Pennisetum glaucum*

1. Introduction

Bajra (*Pennisetum glaucum* L.), commonly known as Pearl Millet, is an essential cereal crop grown primarily in the arid and semi-arid regions of Africa and Asia. This resilient crop is known for its ability to thrive in harsh environmental conditions such as low rainfall, high temperatures, and poor soils, making it a vital food source for millions of people in these areas. Bajra is highly nutritious, containing carbohydrates (67%), protein (11.6%), and minerals (2.7%), which make it an important dietary staple for both humans and livestock (Tejagouda, 2012). It is particularly significant in regions with limited water resources, where its drought-tolerant nature ensures food security and sustenance. However, despite its hardiness, Bajra productivity is often constrained by factors such as weed competition, which can substantially reduce crop yield.

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Weed control in Bajra cultivation is commonly achieved through the use of herbicides. Herbicides offer a convenient and effective means of managing weed populations, which are known to reduce yields by up to 50% in some regions (Dobrovoljskiy and Grishina, 1985). However, the increased reliance on herbicides in modern agriculture raises concerns regarding their potential impact on soil health and microbial communities. Herbicides are designed to target and control weeds, but their application may also affect non-target organisms, particularly the beneficial microorganisms present in the soil. Soil microorganisms, including nitrogen-fixing bacteria, mycorrhizal fungi, and other beneficial microflora, play a crucial role in nutrient cycling, organic matter decomposition, and overall plant health. These microorganisms contribute to plant growth by enhancing nutrient uptake, suppressing soilborne pathogens, and improving soil structure. Any disturbance in their populations due to herbicide application can adversely affect soil fertility, plant growth, and crop yield (Ayansina *et al*., 2003).

The residual effects of herbicides on soil microflora, particularly beneficial microorganisms, remain an area of concern in agricultural research. Some herbicides, especially those with low biodegradability, can persist in the soil for extended periods, leading to long-term alterations in microbial diversity and activity. These herbicide residues may interfere with the natural soil processes, resulting in reduced microbial populations or shifts in microbial community composition. Such changes can negatively influence soil health, nutrient cycling, and the availability of essential nutrients for plants (Ashim *et al*., 2008). Furthermore, herbicides may disrupt the balance between beneficial and harmful microorganisms, potentially leading to the dominance of pathogenic microbes that can further hinder plant growth (Amakiri, 1982).

The present study focuses on the impact of herbicides on beneficial soil microflora in the rhizosphere of Bajra. Specifically, it aims to evaluate how residual herbicide toxicity affects the populations of beneficial microorganisms that are critical for soil fertility and plant health. By investigating the effects of herbicides on the microbial diversity and activity in the Bajra rhizosphere, the study seeks to contribute to a better understanding of the long-term consequences of herbicide use on soil microbial communities. The findings will provide valuable insights into how herbicide-induced changes in soil microflora can affect Bajra growth, nutrient availability, and crop productivity. Ultimately, this research aims to support the development of more sustainable herbicide management practices that minimize harm to beneficial microorganisms and enhance overall soil health, ensuring the continued productivity of Bajra in arid and semi-arid regions (Hutsch, 2001).

2. Material and Methods

2.1 Study site and experimental design

A field experiment was conducted to investigate the residual effects of herbicides on soil microbial communities, including beneficial microflora, and their subsequent influence on the growth and yield of *Pennisetum glaucum* L. (Bajra) under rainfed conditions during the rainy season. The experimental site was part of a long-term cropping sequence, wherein various herbicidal treatments were applied to a preceding chickpea (*Cicer arietinum* L.) crop. The objective was to assess the residual impact of these herbicides on the

rhizospheric soil microbiota and subsequent crop performance.

The study employed a randomized complete block design (RCBD) comprising 18 treatments and three replications. Treatments included both pre-emergence and post-emergence herbicides applied to the chickpea crop. Pre-emergence herbicides were administered on the day of sowing, whereas post-emergence herbicides were applied 20 days after sowing (DAS).

Each plot measured 3 m × 4 m, with row spacing maintained at 45 cm and intra-row spacing at 15 cm. The hybrid variety ICTP-8230 of Bajra was sown in all experimental plots following standard agronomic practices. The treatment details consisting of mode of application and dosage are provided in Table 1.

2.2 Herbicide Treatments

Herbicidal treatments included both individual and combined formulations to simulate practical field conditions. Table 1 summarizes the herbicides employed, their application stages (pre- or post-emergence), and dosages. These treatments were selected to reflect real-world usage patterns and to assess the persistence and subsequent effects of herbicidal residues in soil.

Table 1: Treatments imposed on previous chickpea crop using different herbicides

Treatments	Herbicides	Mode / method of application	Dosage
1	Pendimethalin	Pre emergence	3.3 ml/l
2	Pendimethalin (xtra formulation)	Pre emergence	2.3 ml/l
3	Chlormuron Ethyl	Pre emergence	7.5 g/l
4	Oxyfluorfen	Pre emergence	2.0 ml/l
5	Quizalofop Ethyl	Post emergent	1.5 ml/1
6	Phenaxoprop Ethyl	Post emergent	0.1 ml/l
7	Propaquizafop Ethyl	Post emergent	1 ml/l
8	Oxyfluorfen	Post emergent	1 ml/l
9	Imazethapyr	Post emergent	1 ml/l
10	Pendimethalin+Quizalofop Ethyl	$T_1 + T_6$	3.3 ml/l + 1.5 ml/1
11	Pendimethalin + Phenaxoprop Ethyl	$T_1 + T_7$	3.3 ml/l + 0.1 ml/l
12	Pendimethalin + Propaquizafop	$T_1 + T_8$	3.3 ml/l + 1 ml/l
13	Pendimethalin + Oxyflorofen	$T_1 + T_9$	3.3 ml/l + 1 ml/l
14	Pendimethalin + Imazethapyr	$T_1 + T_{10}$	3.3 ml/l + 1ml/l
15	Weedy check	Weedy check	Control
16	Weed free (Hand weeding)	Herbicides were not imposed	Hand weeding
17	Pendimethalin + Intercultivation	Pre emergence + Intercultivation	3.3 ml/l + intra cultivation
18	Valore -32	Pre emergence	Pendimethalin 3.3 ml + Imazethapyr 2.0 ml was added to l liter of water and the volume was made to 10 liters

Source: Raghavendra, K. S. (2013)

2.3 Soil sampling and physicochemical analysis

Soil samples were collected at four time points: before sowing of Bajra, and at 20, 45, and 60 DAS, as well as at crop harvest. Samples were obtained from the rhizosphere zone at a depth of 0–15 cm using a soil auger, composited, and processed for analysis.

Soil pH was determined in a 1:2.5 soil-to-water suspension using a glass electrode pH meter. Electrical conductivity (EC) was measured using a conductivity bridge, following the protocol outlined by Jackson (1967).

2.4 Enumeration of soil microbial populations

Total soil microbial populations, including key beneficial microorganisms such as *Azotobacter*, *Azospirillum*, and phosphate solubilizing microorganisms (PSM), were assessed using the standard serial dilution and plating technique on selective culture media. General bacterial populations were enumerated on nutrient agar, while specific microbial groups were isolated and quantified using their respective selective media. *Azotobacter* was cultured on Waksman No. 77 medium (Allen, 1953), *Azospirillum* was grown on nitrogen-free malate medium, and PSM were isolated using Pikovskaya's agar medium (Bunt Rovira, 1955). After inoculation, plates were incubated under optimal laboratory conditions, and microbial colonies were counted and expressed as colony-forming units (CFU) per gram of dry soil.

2.5 Statistical analysis

All collected data, including microbial counts and soil chemical parameters, were subjected to analysis of variance (ANOVA) using the statistical procedure described by Gomez and Gomez (1984). The F-test was applied to determine the significance of treatment effects at a probability level of $P \leq 0.05$. Wherever applicable, treatment means were compared using the least significant difference (LSD) test to determine statistically significant differences among treatments.

3. Results and Discussion

3.1 Effect of herbicides on *Azotobacter* population in Bajra rhizosphere

The application of herbicides significantly affected *Azotobacter* populations in the rhizospheric soil of *Pennisetum glaucum* across various growth stages (Table 2). Across all treatments, the population of *Azotobacter* consistently peaked at 60 days after sowing (DAS), suggesting enhanced microbial activity during this phase.

Among pre-emergence herbicides, Pendimethalin consistently supported the highest *Azotobacter* populations across all stages, with a maximum of 4.40×10^4 cfu g^{-1} soil at 60 DAS, whereas Oxyfluorfen showed the most suppressive effects (2.84×10^4 cfu g^{-1}). Post-emergence treatments followed a similar trend: Phenaxoprop-ethyl favored microbial proliferation (4.50×10^4 cfu g^{-1}), while Imazethapyr exhibited inhibitory effects (2.84×10^4 cfu g^{-1}). Sequential applications of Pendimethalin + Propaquizafop-ethyl consistently maintained higher microbial counts, peaking at 4.48×10^4 cfu g^{-1} at 60 DAS. Conversely, Pendimethalin + Imazethapyr reduced the population to 2.79×10^4 cfu g^{-1}.

Table 2: Residual effect of herbicides on *Azotobacter* population at different growth stages of Bajra

Treatments	× 10^4 cfu g^{-1} of soil				
	Before sowing	20 DAS	45 DAS	60 DAS	At harvest
T_1 - Pendimethalin (PRE)	2.02	2.68	2.94	4.40	3.42
T_2 - Pendimethalin (Xtra formulation) (PRE)	1.81	2.57	2.73	4.26	3.30
T_3 - Chlormuron Ethyl (PRE)	1.88	2.56	2.82	4.01	3.11
T_4 -Oxyfluorfen (PRE)	1.49	2.15	2.31	2.84	2.43
T_5 - Quizalofop Ethyl (POE)	1.91	2.60	2.79	4.27	3.35
T_6 - Phenaxoprop Ethyl (POE)	2.06	2.70	2.91	4.50	3.39
T_7 - Propaquizafop Ethyl (POE)	1.81	2.55	2.75	4.37	3.48
T_8 -Oxyfluorfen (POE)	1.53	2.20	2.36	2.90	2.50
T_9 - Imazethapyr (POE)	1.49	2.10	2.26	2.84	2.43
T_{10} - Pendimethalin + Quizalofop Ethyl	1.96	2.56	2.85	4.35	3.27
T_{11} - Pendimethalin + Phenaxoprop Ethyl	1.79	2.51	2.78	4.30	3.32
T_{12} - Pendimethalin + Propaquizafop Ethyl	2.11	2.66	2.97	4.48	3.45
T_{13} - Pendimethalin + Oxyfluorfen	1.60	2.57	2.39	2.95	2.55
T_{14} - Pendimethalin + Imazethapyr	1.45	2.16	2.22	2.79	2.38
T_{15} - Weedy check (WC)	1.88	2.47	2.61	4.22	3.24
T_{16} - Weed free check (WF)	2.26	2.80	3.10	4.65	3.57
T_{17} - Pendimethalin + Intercultivation (IC)	1.85	2.21	2.63	4.21	3.32
T_{18} - Valore-32	1.85	2.39	2.61	4.11	3.23
S.Em±	0.05	0.03	0.04	0.04	0.03
C.D at 5%	0.14	0.08	0.11	0.12	0.10

Note: DAS = Days after sowing, NS = Non significant, PRE = Pre-emergence herbicide, POE = Post-emergence herbicide

Following 60 DAS, a decline in population was observed at harvest. However, the relative trends persisted, with Pendimethalin and Phenaxoprop-ethyl continuing to sustain the highest *Azotobacter* counts and Imazethapyr remaining the most inhibitory.

3.2 Effect of herbicides on *Azospirillum* population in Bajra rhizosphere

Azospirillum populations also demonstrated significant sensitivity to herbicidal influence (Table 3). Population densities peaked at 60 DAS across all treatments, indicating this stage as optimal for *Azospirillum* proliferation, likely due to heightened root exudation and favorable soil conditions.

Pendimethalin once again emerged as the most supportive pre-emergence herbicide, with the *Azospirillum* population reaching 4.38 × 10^6 cfu g^{-1} soil at 60 DAS. Oxyfluorfen, in contrast, suppressed microbial abundance (2.86 × 10^6 cfu g^{-1}). Among post-emergence herbicides, Phenaxoprop-ethyl resulted in the highest count (4.36 × 10^6 cfu g^{-1}), while Imazethapyr was consistently detrimental (2.82 × 10^6 cfu g^{-1}).

Table 3: Residual effect of herbicides on *Azospirillum* population at different growth stages of Bajra

Treatments	× 10^6 cfu g^{-1} of soil				
	Before sowing	20 DAS	45 DAS	60 DAS	At harvest
T_1 - Pendimethalin (PRE)	2.03	2.30	3.00	4.38	3.94
T_2 - Pendimethalin (Xtra formulation) (PRE)	1.86	2.19	2.83	4.28	3.82
T_3 - Chlormuron Ethyl (PRE)	1.85	2.18	2.70	4.11	3.65
T_4 - Oxyfluorfen (PRE)	0.96	1.45	2.04	2.86	2.48
T_5 - Quizalofop Ethyl (POE)	1.80	2.25	2.90	4.26	3.84
T_6 - Phenaxoprop Ethyl (POE)	2.05	2.36	3.14	4.36	3.96
T_7 - Propaquizafop Ethyl (POE)	1.88	2.26	2.95	4.24	3.84
T_8 -Oxyfluorfen (POE)	1.02	1.49	2.18	2.90	2.50
T_9 - Imazethapyr (POE)	0.98	1.16	2.13	2.82	2.36
T_{10} - Pendimethalin + Quizalofop Ethyl	1.80	2.21	2.94	4.22	3.86
T_{11} - Pendimethalin + Phenaxoprop Ethyl	1.68	2.22	2.91	4.24	3.82
T_{12} - Pendimethalin + Propaquizafop Ethyl	1.96	2.31	3.20	4.34	4.00
T_{13} - Pendimethalin + Oxyfluorfen	1.12	1.50	2.15	2.95	2.64
T_{14} - Pendimethalin + Imazethapyr	1.08	1.18	2.07	2.79	2.43
T_{15} - Weedy check (WC)	1.76	2.18	2.90	4.12	3.75
T_{16} - Weed free check (WF)	2.22	2.47	3.35	4.48	4.15
T_{17} - Pendimethalin + Intercultivation (IC)	1.88	2.14	2.80	4.18	3.70
T_{18} - Valore-32	1.94	2.16	2.88	4.20	3.69
S.Em±	0.05	0.03	0.05	0.03	0.04
C.D at 5%	0.16	0.09	0.14	0.09	0.11

Note: DAS = Days after sowing, NS = Non significant, PRE = Pre-emergence herbicide, POE = Post-emergence herbicide

Sequential application of Pendimethalin + Propaquizafop-ethyl supported the highest population (4.34 × 10^6 cfu g^{-1}), while Pendimethalin + Imazethapyr was the least favorable (2.79 × 10^6 cfu g^{-1}).

At harvest, populations declined slightly, in line with senescing roots and declining nutrient levels. However, relative treatment efficacy remained constant, reaffirming the safety of Pendimethalin and Phenaxoprop-ethyl.

3.4 Effect of herbicides on phosphate solubilizing bacteria (PSB) in Bajra rhizosphere

Phosphate solubilizing bacteria (PSB) populations followed a similar trend (Table 4), with maximum proliferation at 60 DAS. Among pre-emergence herbicides, Pendimethalin again yielded the highest population (4.01 × 10^3 cfu g^{-1}), while Oxyfluorfen was least favorable (2.77 × 10^3 cfu g^{-1}). Phenaxoprop-ethyl facilitated the highest PSB counts among post-emergence herbicides (4.20 × 10^3 cfu g^{-1}), whereas Imazethapyr markedly inhibited PSB (2.65 × 10^3 cfu g^{-1}).

Sequentially, Pendimethalin + Propaquizafop-ethyl promoted the highest PSB count (4.11×10^3 cfu g^{-1}), whereas Pendimethalin + Imazethapyr had a suppressive effect (2.64×10^3 cfu g^{-1}).

At harvest, a reduction in microbial activity was evident, yet the relative performance of the treatments remained consistent, emphasizing the persistent effects of herbicide combinations on microbial ecology.

The results clearly indicate that herbicide applications substantially influence beneficial microflora in the rhizosphere of *Pennisetum glaucum*, with Pendimethalin and Phenaxoprop-ethyl showing minimal to no adverse effects on *Azotobacter*, *Azospirillum*, and PSB populations. In contrast, Oxyfluorfen and particularly Imazethapyr consistently demonstrated microbial suppressiveness, regardless of timing or combination. This variation in microbial response aligns with previous reports that certain herbicides, depending on their chemical nature, persistence, and mode of action, can selectively inhibit or promote microbial populations (Cycoń

Table 4: Residual effect of herbicides on phosphate solubilizing bacteria (PSB) population at different growth stages of Bajra

Treatments	× 10^3 cfu g^{-1} of soil				
	Before sowing	20 DAS	45 DAS	60 DAS	At harvest
T_1 - Pendimethalin (PRE)	1.48	2.18	2.66	4.01	3.30
T_2 - Pendimethalin (Xtra formulation) (PRE)	1.25	2.08	2.48	3.77	3.14
T_3 - Chlormuron Ethyl (PRE)	1.25	1.86	2.54	3.86	2.96
T_4 - Oxyfluorfen (PRE)	1.07	1.15	2.07	2.77	2.48
T_5 - Quizalofop Ethyl (POE)	1.29	2.14	2.54	4.08	3.28
T_6 - Phenaxoprop Ethyl (POE)	1.60	2.25	2.65	4.20	3.33
T_7 - Propaquizafop Ethyl (POE)	1.36	2.15	2.57	4.02	3.16
T_8 -Oxyfluorfen (POE)	1.11	1.27	2.12	2.80	2.53
T_9 - Imazethapyr (POE)	1.04	1.25	2.10	2.65	2.47
T_{10} - Pendimethalin + Quizalofop Ethyl	1.30	2.23	2.48	3.99	3.30
T_{11} - Pendimethalin + Phenaxoprop Ethyl	1.18	2.19	2.64	3.86	3.27
T_{12} - Pendimethalin + Propaquizafop Ethyl	1.41	2.35	2.75	4.11	3.40
T_{13} - Pendimethalin + Oxyfluorfen	1.18	1.30	2.26	2.72	2.54
T_{14} - Pendimethalin + Imazethapyr	1.04	1.27	2.11	2.64	2.47
T_{15} - Weedy check (WC)	1.18	2.15	2.51	3.89	3.25
T_{16} - Weed free check (WF)	1.85	2.46	2.85	4.35	3.51
T_{17} - Pendimethalin + Intercultivation (IC)	1.23	2.14	2.43	4.06	3.18
T_{18} - Valore-32	1.22	2.15	2.47	4.04	3.19
S.Em±	0.08	0.03	0.03	0.04	0.03
C.D at 5%	0.23	0.09	0.08	0.11	0.09

Note: DAS = Days after sowing, NS = Non significant, PRE = Pre-emergence herbicide, POE = Post-emergence herbicide

and Piotrowska-Seget, 2015; Walia *et al*., 2017). The superior microbial performance at 60 DAS across all treatments is likely attributed to increased root biomass, exudation, and active rhizospheric interactions (Mishra *et al*., 2013).

Importantly, the sequential combination of Pendimethalin + Propaquizafop-ethyl emerged as the most microbiologically favorable treatment, supporting high populations across all three microbial groups, thus suggesting its compatibility with sustainable crop management strategies. In contrast, the Pendimethalin + Imazethapyr combination consistently recorded the lowest microbial counts, raising concerns about its long-term impact on soil health. These findings underscore the necessity of selecting herbicides not solely based on weed control efficacy, but also on their ecological compatibility with soil microbial communities vital to nutrient cycling and plant growth promotion.

4. Conflicts of interests

Authors declare that there is no conflict of interest exists.

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