



RESEARCH ARTICLE

Soil biological health under integrated weed management strategies in grain cowpea (*Vigna unguiculata* (L.) Walp)

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Abstract

Soil biological indicators such as microbial population and enzyme activities are highly sensitive to chemical weed control and serve as early signals of soil health. Integrated weed management (IWM) with low-dose, high-efficiency herbicides is considered to be environmentally acceptable; however, its impact on soil biological functioning is poorly understood. In the present study, the influence of IWM, comprising pre- and post-emergence herbicides, wheel hoe weeding and hand weeding on soil microbial population and enzymatic activities was evaluated for 2 seasons in grain cowpea. Results indicated that all herbicide applications significantly reduced bacterial, fungal and actinomycete populations temporarily up to 15 days after spraying (DASP), but this reduction was more intense under chlorimuron-ethyl and haloxyfop-R-methyl treatments. Microbial count gradually increased over time as herbicide concentrations declined in the soil, reaching the initial levels by 30 DASP. Dehydrogenase, β -glucosidase and acid phosphatase activities decreased shortly after the application of the herbicides but returned to pre-application levels by 30 DASP, indicating soil resilience and microbial adaptation. However, hand weeding and untreated weedy plots showed higher microbial and enzymatic activities due to increased organic inputs and reduced chemical stress. Among the herbicides evaluated, diclosulam and pretilachlor were identified as the least disruptive to soil biological health. The results indicated that although herbicides may temporarily suppress soil biological activity, the soil system shows strong potential for recovery under integrated weed management practices.

Keywords: acid phosphatase; β -glucosidase; dehydrogenase; herbicides; soil enzymes; soil microbes

Introduction

Soil microorganisms and the enzymes they produce are sensitive indicators of soil quality because they show rapid responses to alterations in management practices and chemical inputs. Among such inputs, herbicides remain indispensable tools in modern agriculture, ensuring effective weed control and enabling higher yields. However, in spite of their agronomic advantages, herbicides have been shown to interfere with natural soil processes and negatively affect non-target soil biota, raising concerns over long-term ecological sustainability (1).

Enzyme activity in soil is considered a useful measure to assess the effect of herbicide applications on soil microbes. Soil enzymes such as dehydrogenase, β -glucosidase and phosphatases are closely related to microbial biomass and activity. They play a crucial role in carbon cycling, redox regulation and phosphorus mineralisation (2). In all living microbial cells, dehydrogenase is present as an intracellular enzyme and it represents the overall microbial activity in soil (3). However, urease is an extracellular enzyme, involved in urea hydrolysis and plays an important role in nitrogen availability for plant growth in the nitrogen cycle. β -glucosidase and acid phosphatase are key soil enzymes that drive

carbon and phosphorus cycling by breaking down cellulose into glucose and hydrolysing organic phosphate esters respectively, making them sensitive indicators of soil biological activity and health (2). Their measurements have been found to be an effective tool for assessing the functionality of soils. Previous studies indicated that herbicides commonly lead to a temporary reduction in enzyme activity, the magnitude of which is determined by the type of herbicide, its persistence and soil conditions (4).

Integrated weed management (IWM), comprising cultural, mechanical and chemical approaches, has gained recognition as a more ecologically balanced strategy that counters the negative impacts attributed to sole reliance on herbicides. Among the herbicides, diclosulam and haloxyfop-R-methyl are low-dose high-efficiency herbicides effective against weeds in grain legumes. Diclosulam is a broad spectrum herbicide controlling all categories of weeds including *Cyperus rotundus* and is recommended in pulses (5). Haloxyfop-R-methyl is another herbicide suited for controlling grass weeds and offered a greater reduction of sedges and dicot weed species in broad-leaf crops including soybean (6). Although several studies were conducted to determine the short-term effects of single herbicides on soil microbial and enzymatic parameters, limited information is available on how complete IWM strategies

impact soil biological indicators throughout the cropping seasons. The present study was conducted with the objective of assessing the impact of integrated weed management practices on soil microbial population and enzyme activities during kharif and rabi seasons. Temporal changes after herbicide application were compared with mechanical and unweeded control to assess soil biological resilience and the relative safety of low-dose high- efficiency herbicides within an IWM framework.

Materials and Methods

The experiment was conducted for 2 seasons during kharif and rabi at the Instructional Farm attached to College of Agriculture, Vellayani, Thiruvananthapuram district, Kerala, India situated at geographical coordinates 8°29'40.9" N latitude and 76°59'14.1" E longitude, at an altitude of 29 m above mean sea level. The kharif crop soil was neutral in reaction with medium organic carbon and high nitrogen, phosphorus and potassium, whereas the rabi crop soil was moderately acidic with medium organic carbon, high nitrogen and phosphorus and medium potassium. The land was prepared adopting state seed bed technique to exhaust tuber reserves of *Cyperus rotundus* followed by shallow tillage and levelling to prepare the seed beds (7). The field was uniformly manured with FYM (20 t ha⁻¹). The cowpea variety DC-15 was raised with 9 weed management treatments laid out in a randomised complete block design with 3 replications. The treatment details of the experiment are given in Table 1. *Rhizobium*-treated seeds were shade-dried for 30 min and dibbled at 2 seeds per hole at 25 × 15 cm spacing. Nutrient dose of 20:30:10 kg N:P₂O₅:K₂O ha⁻¹ was adopted for nutrient management as per KAU package of practices recommendations: Crops (8).

Spray volume used in the study was 500 L ha⁻¹ and herbicides were sprayed using a hand operated sprayer of 1 L capacity fitted with flat fan nozzle. In treatments T₁ and T₇, pre-emergence herbicide application of diclosulam and pretilachlor was

carried out on the next day of sowing. In T₂, post-directed application of the herbicide chlorimuron-ethyl was done at 20 DAS by directing the spray towards the weeds. In treatment T₃, post-emergence herbicide application of haloxyfop-R-methyl was done at 20 DAS. Wheel hoe weeding was done at 25 DAS in treatment T₄ and at 40 DAS in T₅ and T₆. In hand weeding plots, manual weeding was done twice, at 20 and 40 DAS. In weedy check plots, no weed management operations were taken up.

Soil samples from a depth of 0–15 cm were collected using a soil auger and composite samples were prepared for estimating the total microbial count of fungi, bacteria and actinomycetes using serial dilution and plating technique (9). The microbial count was estimated before spraying; at 15 and 30 DAS and expressed in cfu g⁻¹ soil. Fungi were cultured on Martin's Rose Bengal agar, bacteria on nutrient agar and actinomycetes on Kenknight's agar medium. The dehydrogenase enzyme activity was determined before spraying and at 15 and 30 DAS as per the procedure described earlier (10) and expressed as µg triphenyl formazon (TPF) hydrolysed g⁻¹ soil 24 hr⁻¹. For estimating β-glucosidase activity, soil was incubated with buffered (pH 6.0) para nitrophenyl β-glucopyranoside and para-nitrophenol released was determined and expressed as µg para-nitrophenol g⁻¹ soil hr⁻¹ (11). For acid phosphatase, soil was incubated with buffered (pH 6.5) para nitrophenyl phosphate tetrahydrate and absorbance of the resultant solution was measured using a spectrophotometer at 410 nm and para-nitrophenol released was expressed as µg para-nitrophenol g⁻¹ soil hr⁻¹ (12).

Statistical analysis

Data analysis was carried out using GRAPES 1.0.0, the statistical software developed by Kerala Agricultural University by performing one-way analysis of variance (ANOVA) to assess treatment effects and means were compared using the least significant difference (LSD) test at $p \leq 0.05$.

Table 1. Effect of weed management practices on total fungal, actinomycetes and bacterial population in soil ($\times 10^5$ CFU g⁻¹ of soil) during kharif season

Treatments	Fungal population			Actinomycetes population			Bacterial population		
	Just before spraying	15 DASP	30 DASP	Just before spraying	15 DASP	30 DASP	Just before spraying	15 DASP	30 DASP
T ₁	3.00 ± 0.00 ^b	1.33 ± 0.58 ^{bc}	3.67 ± 0.58 ^a	9.67 ± 2.08	4.33 ± 0.58 ^b	10.33 ± 1.53	28.00 ± 2.00 ^{bcd}	13.00 ± 1.00 ^b	32.33 ± 1.53 ^{ab}
T ₂	4.33 ± 0.58 ^a	0.67 ± 0.58 ^d	2.67 ± 0.58 ^{bc}	11.67 ± 2.52	2.33 ± 0.58 ^c	10.33 ± 1.15	34.00 ± 1.00 ^{ab}	11.33 ± 2.52 ^b	28.00 ± 1.73 ^c
T ₃	4.00 ± 0.00 ^a	1.00 ± 0.00 ^{cd}	3.00 ± 0.00 ^{ab}	11.67 ± 3.06	3.33 ± 0.58 ^{bc}	10.67 ± 1.53	33.33 ± 1.15 ^{abc}	11.67 ± 1.53 ^b	29.67 ± 1.53 ^{abc}
T ₄	2.67 ± 0.58 ^b	1.67 ± 0.58 ^b	3.67 ± 0.58 ^a	10.00 ± 2.00	4.67 ± 0.58 ^b	11.00 ± 3.00	27.00 ± 3.61 ^d	12.67 ± 1.53 ^b	32.00 ± 3.46 ^{ab}
T ₅	4.00 ± 0.00 ^a	1.00 ± 0.00 ^{cd}	2.00 ± 0.00 ^c	11.33 ± 1.53	2.33 ± 0.58 ^c	10.67 ± 1.53	33.33 ± 1.15 ^{abc}	10.67 ± 1.53 ^b	28.33 ± 1.53 ^c
T ₆	4.33 ± 0.58 ^a	0.67 ± 0.58 ^d	3.00 ± 0.00 ^{ab}	11.67 ± 3.06	3.33 ± 0.58 ^{bc}	11.00 ± 1.00	35.33 ± 9.29 ^a	11.67 ± 3.51 ^b	29.33 ± 2.08 ^{bc}
T ₇	2.67 ± 0.58 ^b	1.33 ± 0.58 ^{bc}	3.33 ± 0.58 ^{ab}	9.67 ± 1.53	3.67 ± 0.58 ^{bc}	11.00 ± 1.00	27.67 ± 1.53 ^{cd}	12.67 ± 2.52 ^b	32.00 ± 2.65 ^{ab}
T ₈	3.00 ± 0.00 ^b	3.00 ± 0.00 ^a	3.67 ± 0.58 ^a	10.67 ± 2.31	11.33 ± 2.08 ^a	12.00 ± 1.00	28.33 ± 1.53 ^{bcd}	32.67 ± 1.15 ^a	33.00 ± 1.73 ^a
T ₉	2.67 ± 0.58 ^b	2.67 ± 0.58 ^a	3.33 ± 0.58 ^{ab}	10.00 ± 1.00	10.33 ± 1.15 ^a	11.00 ± 1.00	28.00 ± 1.00 ^{bcd}	31.67 ± 0.58 ^a	32.00 ± 1.00 ^{ab}
SEm (±)	0.26	0.20	0.25	0.80	0.54	0.61	2.04	1.18	1.16
CD (0.05)	0.780	0.600	0.750	NS	1.630	NS	6.110	3.550	3.480

T₁- Pre-emergence application of diclosulam at 12.5 g ha⁻¹; T₂- Post directed application of chlorimuron-ethyl at 9 g ha⁻¹ at 20 DAS; T₃- Post-emergence application of haloxyfop-R-methyl at 100 g ha⁻¹ at 20 DAS; T₄-T₁ followed by wheel hoe weeding at 25 DAS; T₅- T₂ followed by wheel hoe weeding at 40 DAS; T₆- T₃ followed by wheel hoe weeding at 40 DAS; T₇- Pre-emergence application of pretilachlor at 0.75 kg ha⁻¹; T₈- Hand weeding twice at 20 and 40 DAS; T₉- Weedy check; DASP- days after spraying.

Values are means of 3 replications; Values with different letters are significantly different from each other at 5 % level of significance.

Results

Status of soil microbial community

Fungal population

The fungal population varied significantly across weed management practices at all sampling stages during both seasons (Tables 1 and 2). At 15 DASP, herbicide-treated plots exhibited a pronounced decline, with the lowest counts in chlorimuron-ethyl and T_6 treatments, whereas the highest population was observed in hand-weeded and weedy-check plots during kharif (3.00×10^5 and 2.67×10^5 CFU g^{-1} soil) and rabi (3.33×10^5 and 3.00×10^5 CFU g^{-1} soil). By 30 DASP, fungal population recovered in diclosulam and diclosulam + hoe-weeding treatments and in hand-weeded plots during kharif, while in rabi the highest counts persisted in hand-weeded and weedy-check plots; chlorimuron-ethyl consistently recorded the lowest fungal population during both seasons.

Actinomycetes population

During both the seasons, the population of actinomycetes did not vary significantly before the application of herbicides. The treatment effects became significant at 15 DASP during both seasons and at 30 DASP during rabi (Table 2). The actinomycetes population during both seasons was the highest in hand weeded and weedy check plots, with 1.13×10^7 and 1.3×10^7 CFU g^{-1} soil respectively. This was followed by diclosulam applied plots, with 4.67×10^6 CFU g^{-1} soil. Similar results were observed during rabi, where hand weeded and weedy check showed the highest population, at 1.40×10^7 and 1.37×10^7 CFU g^{-1} soil respectively, while diclosulam applied plots recorded next higher population. The lowest population was observed in chlorimuron-ethyl treated plots, recording 1.3×10^7 CFU g^{-1} , on par with T_3 and T_6 .

Bacterial population

During both seasons, significant differences were observed at all 3 sampling stages. At 15 DASP, hand weeded and weedy check plots showed the highest bacterial population in kharif (3.27×10^8 and 3.17×10^8 CFU g^{-1}) and rabi (2.07×10^8 and 2.00×10^8 CFU g^{-1}) respectively, with other weed management options recording

significantly lower values. A lower bacterial population was recorded in chlorimuron-ethyl treated plots alone and with wheel hoe weeding during both the seasons, which were comparable to all IWM practices excluding chlorimuron-ethyl. At 30 DASP, hand weeding retained the highest bacterial population (3.30×10^8 CFU g^{-1}) which was on par with all the IWM options except chlorimuron-ethyl which recorded the lowest during kharif. However, in rabi, diclosulam plots recorded the highest count of 2.30×10^8 CFU g^{-1} which was statistically at par to diclosulam and pretilachlor applied plots, while chlorimuron-ethyl application resulted in the lowest population.

Soil enzyme activities

Dehydrogenase

Dehydrogenase activity differed significantly among the weed management practices at each sampling stage during both seasons (Tables 3 and 4). At 15 DASP, it showed a steep decline in herbicide-treated plots and the lowest activity was observed with chlorimuron-ethyl application (T_2 and T_5), while hand-weeded and weedy-check plots (T_8 and T_9) consistently retained the highest activity throughout both seasons. At 30 DASP, partial recovery in enzyme activity was noticed and higher activities were recorded in IWM practices involving diclosulam and pretilachlor application, while chlorimuron-ethyl plots (T_2 and T_5) invariably exhibited the lowest activity during both seasons.

β -glucosidase

β -glucosidase activity was significantly different among the treatments in all the sampling stages for the 2 seasons (Tables 3 and 4). At 15 DASP, the enzyme activity decreased sharply in the herbicide-applied plots, with the lowest values observed with chlorimuron-ethyl treatments (T_2 and T_5), whereas hand weeded and unweeded plots consistently maintained the highest values during both seasons. β -glucosidase activity partially recovered at 30 DASP, with higher activities recorded in IWM with diclosulam, T_8 and T_7 for both seasons, while chlorimuron-ethyl plots (T_2 , T_5) again recorded the least values for β -glucosidase.

Table 2. Effect of weed management practices on total fungal, actinomycetes and bacterial population in soil ($\times 10^5$ CFU g^{-1} of soil) during rabi season

Treatments	Fungal population			Actinomycetes population			Bacterial population		
	Just before spraying	15 DASP	30 DASP	Just before spraying	15 DASP	30 DASP	Just before spraying	15 DASP	30 DASP
T_1	2.67 ± 0.58^{bc}	1.67 ± 0.58^{bc}	3.00 ± 0.00^{ab}	13.67 ± 0.58	7.33 ± 1.53^b	14.00 ± 3.61^{ab}	19.67 ± 1.53^{bc}	11.00 ± 2.00^b	23.00 ± 1.00^a
T_2	3.00 ± 0.00^{abc}	0.67 ± 0.58^d	2.00 ± 0.00^c	15.33 ± 1.53	5.00 ± 0.00^d	11.33 ± 2.52^c	24.67 ± 1.15^{ab}	8.00 ± 2.00^c	18.33 ± 1.53^{cd}
T_3	3.00 ± 0.00^{abc}	1.00 ± 0.00^{cd}	2.33 ± 0.58^{bc}	14.67 ± 0.58	5.67 ± 1.15^{cd}	12.00 ± 3.00^{bc}	24.00 ± 0.00^{abc}	8.67 ± 1.53^{bc}	19.00 ± 1.00^{bcd}
T_4	2.33 ± 0.58^c	2.00 ± 0.00^b	3.00 ± 1.00^{ab}	13.67 ± 0.58	7.67 ± 1.53^b	14.67 ± 2.52^a	20.00 ± 3.00^{bc}	10.33 ± 1.15^{bc}	22.67 ± 2.31^a
T_5	3.67 ± 0.58^a	1.00 ± 0.00^{cd}	2.33 ± 0.58^{bc}	14.67 ± 1.15	5.00 ± 0.00^d	12.67 ± 2.08^{abc}	26.00 ± 7.00^a	8.00 ± 3.00^c	18.00 ± 2.00^d
T_6	3.00 ± 0.00^{abc}	1.33 ± 0.58^{bcd}	2.67 ± 0.58^{abc}	15.00 ± 1.00	5.33 ± 0.58^d	12.33 ± 1.53^{bc}	24.00 ± 0.00^{abc}	9.00 ± 0.00^{bc}	19.33 ± 1.15^{bcd}
T_7	2.33 ± 0.58^c	2.00 ± 0.00^b	2.67 ± 0.58^{abc}	13.33 ± 1.15	7.00 ± 0.00^{bc}	13.67 ± 1.15^{ab}	19.33 ± 2.08^c	10.00 ± 1.00^{bc}	21.67 ± 1.15^{ab}
T_8	3.33 ± 0.58^{ab}	3.33 ± 0.58^a	3.33 ± 0.58^a	13.67 ± 0.58	14.00 ± 0.00^a	14.67 ± 0.58^a	20.00 ± 1.00^{bc}	20.67 ± 1.53^a	21.33 ± 2.52^{abc}
T_9	3.00 ± 0.00^{abc}	3.00 ± 0.00^a	3.33 ± 0.58^a	13.33 ± 0.58	13.67 ± 0.58^a	13.67 ± 0.58^{ab}	19.33 ± 1.15^c	20.00 ± 1.00^a	20.67 ± 3.21^{abcd}
SEm ($\pm$)	0.26	0.24	0.26	0.56	0.48	0.72	1.67	0.99	1.11
CD (0.05)	0.78	0.71	0.78	NS	1.450	2.150	5.000	2.970	3.330

T_1 - Pre-emergence application of diclosulam at $12.5 g ha^{-1}$; T_2 - Post directed application of chlorimuron-ethyl at $9 g ha^{-1}$ at 20 DAS; T_3 - Post-emergence application of haloxyfop-R-methyl at $100 g ha^{-1}$ at 20 DAS; T_4 - T_1 followed by wheel hoe weeding at 25 DAS; T_5 - T_2 followed by wheel hoe weeding at 40 DAS; T_6 - T_3 followed by wheel hoe weeding at 40 DAS; T_7 - Pre-emergence application of pretilachlor at $0.75 kg ha^{-1}$; T_8 - Hand weeding twice at 20 and 40 DAS; T_9 - Weedy check; DASP- days after spraying.

Values are means of 3 replications; Values with different letters are significantly different from each other at 5 % level of significance.

Table 3. Effect of weed management practices on enzyme activity in soil during kharif season, $\mu\text{g TPF g}^{-1} 24 \text{ hr}^{-1}$

Treatments	Dehydrogenase activity			β -glucosidase activity			Acid phosphatase activity		
	Just before spraying	15 DASP	30 DASP	Just before spraying	15 DASP	30 DASP	Just before spraying	15 DASP	30 DASP
T ₁	4.85 $\pm$ 0.15 ^{bc}	3.81 $\pm$ 0.04 ^{bc}	5.24 $\pm$ 0.53 ^a	32.20 $\pm$ 1.45 ^d	24.94 $\pm$ 0.21 ^b	37.28 $\pm$ 6.44 ^a	44.72 $\pm$ 1.05 ^{cd}	38.87 $\pm$ 0.41 ^{bc}	49.22 $\pm$ 6.78 ^a
T ₂	6.83 $\pm$ 1.10 ^a	2.74 $\pm$ 0.02 ^d	3.14 $\pm$ 0.19 ^c	42.65 $\pm$ 9.47 ^a	20.35 $\pm$ 0.12 ^b	28.73 $\pm$ 1.92 ^c	51.01 $\pm$ 3.97 ^{abc}	31.61 $\pm$ 0.03 ^d	32.22 $\pm$ 1.80 ^c
T ₃	6.72 $\pm$ 0.92 ^a	3.19 $\pm$ 0.02 ^{cd}	4.77 $\pm$ 0.07 ^a	40.91 $\pm$ 5.95 ^{ab}	21.18 $\pm$ 0.13 ^b	30.08 $\pm$ 1.71 ^{bc}	52.38 $\pm$ 5.94 ^{ab}	34.03 $\pm$ 0.08 ^{cd}	37.94 $\pm$ 1.95 ^{bc}
T ₄	5.05 $\pm$ 0.14 ^{bc}	3.84 $\pm$ 0.05 ^{bc}	5.62 $\pm$ 1.33 ^a	33.33 $\pm$ 1.25 ^{bcd}	24.77 $\pm$ 0.20 ^b	37.80 $\pm$ 7.56 ^a	45.82 $\pm$ 0.58 ^{bcd}	38.54 $\pm$ 0.37 ^{bcd}	50.40 $\pm$ 9.09 ^a
T ₅	6.14 $\pm$ 0.24 ^{ab}	3.08 $\pm$ 0.02 ^{cd}	3.74 $\pm$ 0.26 ^{bc}	40.20 $\pm$ 4.67 ^{abc}	20.11 $\pm$ 0.12 ^b	29.15 $\pm$ 1.87 ^c	49.44 $\pm$ 2.13 ^{abcd}	32.45 $\pm$ 0.04 ^{cd}	33.56 $\pm$ 1.90 ^c
T ₆	7.27 $\pm$ 1.94 ^a	3.77 $\pm$ 0.04 ^c	4.85 $\pm$ 0.01 ^a	41.31 $\pm$ 6.70 ^a	21.60 $\pm$ 0.13 ^b	31.85 $\pm$ 1.06 ^{abc}	54.73 $\pm$ 10.05 ^a	32.86 $\pm$ 0.05 ^{cd}	38.30 $\pm$ 1.92 ^{bc}
T ₇	4.33 $\pm$ 0.14 ^c	3.64 $\pm$ 0.02 ^c	5.09 $\pm$ 0.28 ^a	30.77 $\pm$ 1.57 ^d	24.34 $\pm$ 0.18 ^b	35.87 $\pm$ 3.59 ^{ab}	46.65 $\pm$ 0.13 ^{bcd}	37.39 $\pm$ 0.27 ^{bcd}	48.12 $\pm$ 4.84 ^a
T ₈	4.85 $\pm$ 0.15 ^{bc}	5.43 $\pm$ 1.55 ^a	5.56 $\pm$ 1.19 ^a	32.48 $\pm$ 1.41 ^{cd}	34.74 $\pm$ 8.20 ^a	36.32 $\pm$ 4.34 ^{ab}	45.37 $\pm$ 0.79 ^{cd}	48.52 $\pm$ 12.47 ^a	51.61 $\pm$ 1.65 ^a
T ₉	4.45 $\pm$ 0.15 ^c	4.68 $\pm$ 0.46 ^{ab}	4.76 $\pm$ 0.08 ^{ab}	30.93 $\pm$ 1.56 ^d	31.44 $\pm$ 4.11 ^a	33.78 $\pm$ 0.50 ^{abc}	42.94 $\pm$ 1.59 ^d	42.34 $\pm$ 4.58 ^{ab}	44.96 $\pm$ 0.81 ^{ab}
SEm ($\pm$)	0.44	0.30	0.34	2.58	1.64	2.18	2.29	2.41	3.10
CD (0.05)	1.317	0.894	1.027	7.734	4.911	6.529	6.851	7.239	9.281

T₁- Pre-emergence application of diclosulam at 12.5 g ha⁻¹; T₂- Post directed application of chlorimuron-ethyl at 9 g ha⁻¹ at 20 DAS; T₃- Post-emergence application of haloxyfop- R- methyl at 100 g ha⁻¹ at 20 DAS; T₄-T₁ followed by wheel hoe weeding at 25 DAS; T₅- T₂ followed by wheel hoe weeding at 40 DAS; T₆- T₃ followed by wheel hoe weeding at 40 DAS; T₇- Pre-emergence application of pretilachlor at 0.75 kg ha⁻¹; T₈- Hand weeding twice at 20 and 40 DAS; T₉- Weedy check.

Values are means of 3 replications; Values with different letters are significantly different from each other at 5 % level of significance.

Table 4. Effect of weed management practices on enzyme activity in soil during rabi season, $\mu\text{g TPF g}^{-1} 24 \text{ hr}^{-1}$

Treatments	Dehydrogenase activity			β -glucosidase activity			Acid phosphatase activity		
	Just before spraying	15 DASP	30 DASP	Just before spraying	15 DASP	30 DASP	Just before spraying	15 DASP	30 DASP
T ₁	4.27 $\pm$ 0.12 ^{bc}	3.00 $\pm$ 0.03 ^{cd}	4.98 $\pm$ 0.80 ^{ab}	31.56 $\pm$ 1.09 ^b	27.29 $\pm$ 0.20 ^{bc}	34.79 $\pm$ 4.21 ^{ab}	35.96 $\pm$ 1.22 ^b	36.17 $\pm$ 0.26 ^{abc}	46.31 $\pm$ 8.37 ^a
T ₂	5.62 $\pm$ 0.58 ^a	1.92 $\pm$ 0.01 ^e	3.97 $\pm$ 0.15 ^c	40.53 $\pm$ 5.77 ^a	22.45 $\pm$ 0.13 ^d	27.30 $\pm$ 1.39 ^d	49.58 $\pm$ 5.35 ^a	28.92 $\pm$ 0.10 ^d	29.03 $\pm$ 1.68 ^c
T ₃	5.67 $\pm$ 0.65 ^a	2.17 $\pm$ 0.01 ^e	4.31 $\pm$ 0.02 ^{abc}	40.26 $\pm$ 5.32 ^a	24.48 $\pm$ 0.15 ^{cd}	29.56 $\pm$ 0.91 ^{bcd}	52.31 $\pm$ 9.75 ^a	31.34 $\pm$ 0.08 ^{cd}	34.75 $\pm$ 1.92 ^{bc}
T ₄	4.16 $\pm$ 0.13 ^{bc}	3.45 $\pm$ 0.08 ^{bc}	4.43 $\pm$ 0.06 ^{abc}	29.86 $\pm$ 1.22 ^b	26.71 $\pm$ 0.19 ^{bcd}	37.37 $\pm$ 9.36 ^a	36.62 $\pm$ 1.20 ^b	35.84 $\pm$ 0.22 ^{abc}	47.73 $\pm$ 11.44 ^a
T ₅	5.33 $\pm$ 0.26 ^{ab}	1.78 $\pm$ 0.01 ^e	3.74 $\pm$ 0.18 ^c	41.72 $\pm$ 7.97 ^a	23.06 $\pm$ 0.14 ^{cd}	28.01 $\pm$ 1.30 ^{cd}	52.80 $\pm$ 10.65 ^a	29.76 $\pm$ 0.10 ^{cd}	30.36 $\pm$ 1.80 ^c
T ₆	6.40 $\pm$ 2.00 ^a	2.58 $\pm$ 0.01 ^{de}	4.98 $\pm$ 0.80 ^{ab}	40.71 $\pm$ 6.09 ^a	23.92 $\pm$ 0.15 ^{cd}	28.20 $\pm$ 1.26 ^{cd}	48.21 $\pm$ 3.64 ^a	30.17 $\pm$ 0.10 ^{cd}	35.10 $\pm$ 1.89 ^{bc}
T ₇	3.92 $\pm$ 0.13 ^c	3.19 $\pm$ 0.04 ^{cd}	4.10 $\pm$ 0.12 ^c	30.22 $\pm$ 1.20 ^b	25.92 $\pm$ 0.17 ^{cd}	33.50 $\pm$ 2.21 ^{abc}	36.99 $\pm$ 1.18 ^b	34.69 $\pm$ 0.10 ^{bcd}	45.08 $\pm$ 5.91 ^a
T ₈	4.33 $\pm$ 0.12 ^{bc}	4.94 $\pm$ 1.47 ^a	5.00 $\pm$ 0.85 ^a	30.13 $\pm$ 1.21 ^b	33.98 $\pm$ 7.26 ^a	34.12 $\pm$ 3.10 ^{abc}	38.03 $\pm$ 1.13 ^b	42.57 $\pm$ 10.75 ^a	45.56 $\pm$ 6.85 ^a
T ₉	4.12 $\pm$ 0.13 ^{bc}	4.18 $\pm$ 0.43 ^{ab}	4.20 $\pm$ 0.08 ^{bc}	29.92 $\pm$ 1.22 ^b	31.04 $\pm$ 4.96 ^{ab}	32.11 $\pm$ 0.63 ^{abcd}	37.61 $\pm$ 1.15 ^b	39.99 $\pm$ 6.72 ^{ab}	40.25 $\pm$ 0.27 ^{ab}
SEm ($\pm$)	0.41	0.28	0.26	2.31	1.55	2.07	2.86	2.29	3.07
CD (0.05)	1.221	0.836	0.787	6.933	4.649	6.193	8.568	6.875	9.208

T₁- Pre-emergence application of diclosulam at 12.5 g ha⁻¹; T₂- Post directed application of chlorimuron-ethyl at 9 g ha⁻¹ at 20 DAS; T₃- Post-emergence application of haloxyfop- R- methyl at 100 g ha⁻¹ at 20 DAS; T₄- T₁ followed by wheel hoe weeding at 25 DAS; T₅- T₂ followed by wheel hoe weeding at 40 DAS; T₆- T₃ followed by wheel hoe weeding at 40 DAS; T₇- Pre-emergence application of pretilachlor at 0.75 kg ha⁻¹; T₈- Hand weeding twice at 20 and 40 DAS; T₉- Weedy check.

Values are means of 3 replications; Values with different letters are significantly different from each other at 5 % level of significance.

Acid phosphatase

Acid phosphatase activity differed significantly among weed management treatments at all sampling stages during both seasons (Tables 3 and 4). At 15 DASP, hand weeding resulted in the highest activity during both seasons and was on par with weedy check, while chlorimuron-ethyl application consistently resulted in the lowest activity and remained comparable with IWM involving haloxyfop- R- methyl application. At 30 DASP, diclosulam, pretilachlor and hand-weeded plots recorded higher acid phosphatase activity during both kharif and rabi seasons and these were statistically comparable to the weedy check. Across both seasons, chlorimuron-ethyl consistently recorded the lowest acid phosphatase activity at 30 DASP.

Discussion

Soil microorganisms drive several biochemical transformations and any disturbance in their population or activity immediately affects soil functioning. Herbicide-based weed management caused marked reductions in bacterial, fungal and actinomycetes population at 15 DASP during both seasons, but counts recovered within 30 days of spraying as herbicide residues dissipated. Similar transient declines followed by rebound have been reported for several herbicide groups, reflecting short-term toxicity during peak residue concentration and subsequent microbial adaptation or degradation (13). Among the evaluated herbicides, chlorimuron-ethyl and haloxyfop-R-methyl caused the strongest suppression at

15 DASP and showed comparatively slower recovery. Chlorimuron-ethyl, a sulfonylurea herbicide, possesses a sulfonylurea bridge and heterocyclic ring system known for high bioactivity at low concentrations. Its mode of action involves ALS inhibition, a pathway present in many soil microorganisms also, explaining their susceptibility (14). Previous studies have shown that sulfonylureas reduce microbial biomass, nitrification and enzyme activity, particularly dehydrogenase and urease, during early residue persistence (15). Application of chlorimuron-ethyl altered microbial community structure, indicating a dosage-dependent influence on living microbial biomass and overall microbial dynamics (16). Haloxyfop-R-methyl, an aryloxyphenoxypropionate herbicide, targets ACCase in grasses, but its lipophilic aromatic ester structure enables strong interaction with microbial cell membranes, causing transient toxicity (17). Hydrolysis converts it into haloxyfop acid, which is less biologically active, explaining the gradual microbial recovery observed.

In contrast, diclosulam and pretilachlor produced lower reductions and faster rebound of microbial population. Diclosulam, though an ALS inhibitor, undergoes rapid microbial degradation into low-toxicity metabolites (18), which likely reduces its impact on soil biota. Earlier studies reported minimal long-term effects of diclosulam on soil enzyme activities and microbial biomass (19). Pretilachlor, a chloroacetamide herbicide, inhibits lipid synthesis in plants but exhibits relatively low toxicity to non-target microbial population due to its moderate persistence and weaker binding affinity for microbial enzymes (20). Fast dissipation through volatilisation, microbial breakdown and sorption also contributes to rapid biological recovery. The observed pattern of early microbial suppression followed by recovery within one month after spraying reflected typical herbicide behaviour, with peak toxicity during high residue levels and reduced impact as microbes degrade or metabolise the compounds (21). Microbial adaptation and the ability to utilise herbicide residues as carbon sources further hasten this recovery (4, 22).

Soil enzyme activities exhibited the same temporal pattern observed for microbial population, with a pronounced decline at 15 DASP followed by recovery at 30 DASP. Dehydrogenase, a key indicator of microbial oxidative metabolism and redox reactions, showed the greatest suppression under chlorimuron-ethyl, followed by haloxyfop-R-methyl, pretilachlor and diclosulam, reflecting the relative toxicity and persistence of these compounds. Sulfonylurea herbicides such as chlorimuron-ethyl can inhibit dehydrogenase activity as their heterocyclic sulfonylurea structure interferes with microbial amino-acid synthesis and respiration (14). Similarly, transient reductions under haloxyfop-R-methyl and pretilachlor are consistent with reports that both post-emergence and pre-emergence herbicides suppress soil metabolic enzymes during early residue persistence (4).

β -glucosidase, which mediates cellulose breakdown and is strongly linked with organic matter turnover, followed a pattern similar to dehydrogenase. Its inhibition at 15 DASP reflects a temporary slowing of carbon cycling processes; in line with findings that herbicides can disrupt C-transforming enzymes shortly after application (23). Acid phosphatase exhibited comparable trends, indicating a short-lived impairment of phosphorus mineralisation pathways. Since these enzymes are predominantly of microbial origin, their early decline corresponds to reduced microbial abundance and suppressed metabolic activity during peak

herbicide concentrations (24, 25). The recovery of all enzyme activities within 1 month after spraying indicated microbial adaptation and degradation of herbicide molecules. Similar rebound patterns in enzyme activity following initial inhibition have been widely documented (26–28). In contrast, untreated and hand-weeded plots consistently maintained higher enzyme activities, owing to greater microbial biomass and organic inputs that stimulate enzyme synthesis (29, 30).

Conclusion

Herbicide-based weed management caused a temporary decline in soil microbial counts and enzyme activities at 15 days after application, with recovery by 30 days as residues dissipated. Chlorimuron-ethyl and haloxyfop-R-methyl imposed the strongest short-term suppression, while diclosulam had the least effect. Hence diclosulam could be recommended as a safe herbicide component for integrated nutsedge management in grain cowpea.

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Authors' contributions

MSRK performed the experiments. SKR and MJ arranged the resources. MSRK and MA wrote the manuscript. PSP, KS, VSS and KNA reviewed the manuscript and AM supervised the study. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: The authors declare that they have no competing interests.

Ethical issues: None

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