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EFFECT OF GLYPHOSATE APPLICATION ON SOIL MICROBIAL POPULATION UNDER ARECANUT PLANTATION

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ABSTRACT

A field experiment was conducted during 2023-24 at a farmer's field in Abbalagere village, Shivamogga taluk, Karnataka, to evaluate the effect of different doses and repeated applications of glyphosate on soil microbial populations under arecanut plantation. The experiment was laid out in a Randomized Complete Block Design with five treatments comprising a control (water spray) and four doses of glyphosate (41% SL) applied at 1.23, 2.05, 2.87 and 3.69 kg a.i. ha^{-1}, with four replications. Glyphosate was applied as foliar spray thrice at two month intervals. Soil samples were collected at 0, 30 and 60 days after application and analysed for bacterial, fungal and actinomycetes populations using the dilution plate count technique. Microbial populations showed no significant variation immediately after application however, a pronounced dose dependent decline was observed at 30 days after application. The highest microbial populations among glyphosate treatments were consistently recorded at 1.23 kg a.i. ha^{-1}, with maximum bacterial (38.27 × 10^6 cfu g^{-1} soil), fungal (29.15 × 10^4 cfu g^{-1} soil) and actinomycetes (15.95 × 10^5 cfu g^{-1} soil) counts at 0 day. In contrast, the lowest populations were observed at the highest dose of 3.69 kg a.i. ha^{-1}, particularly after repeated applications, recording bacterial (11.00 × 10^6 cfu g^{-1} soil), fungal (4.19 × 10^4 cfu g^{-1} soil) and actinomycetes (1.39 × 10^5 cfu g^{-1} soil) populations at 30 days. Partial recovery of microbial populations was observed by 60 days after application mainly at lower doses, whereas higher doses exhibited sustained suppression. The study demonstrates that repeated application of glyphosate at higher rates adversely affects soil microbial populations under arecanut plantation, emphasizing the need for judicious herbicide use to maintain soil biological health.

***Key words*:** Glyphosate, Arecanut plantation, Microbial population, Soil health

Introduction

Pesticides play a pivotal role in crop protection and production. Crop loss due to pests is the biggest challenge in agriculture sector facing today. Pesticides help farmers to overcome work costs by diminishing the measure of time required to control weeds and pests from fields. Pesticides are chemicals to control a variety of pests that can damage crops and livestock and reduce farm productivity. The pesticides cover a wide range of compounds including insecticides, fungicides, herbicides, rodenticides, molluscicides, nematicides, plant growth regulators and others (Bhatt *et al.*, 2021). Herbicides account for 42 per cent, insecticides 27 per cent, fungicides 22 per cent and disinfectants and other agrochemicals 9 per cent of global pesticide sales whereas, in India 76 per cent of the pesticide used is insecticide and use of herbicides and fungicides is correspondingly less heavy (Mathur, 1999).

Herbicide usage has become essential in modern intensive agriculture to achieve high yield and minimize losses due to weeds. In India, the demand for herbicides is rising sharply and is expected to double in the coming years due to labor shortages and the cost effectiveness of chemical weed control. Globally, herbicides account for about 44 per cent of total agrochemicals, while in India they represent nearly 30 per cent of usage (Sondhia, 2014). Herbicides are organic compounds designed to control unwanted vegetation, but their persistence in soil raises environmental concerns. A herbicide is considered persistent if it remains in the soil in its original or closely related phytotoxic forms even after its intended purpose is achieved (Sankaran *et al.*, 1993; Sondhia, 2014). The residues of persistent herbicides can affect soil physicochemical properties, microbial populations and overall soil health, highlighting the need for careful management and monitoring of herbicide use in agricultural systems.

Among production constraints in arecanut cultivation is weed infestation. Weed infestation is more prevalent in sole stands of arecanut compared to multi-story cropping systems, where the available space is optimally utilized. Glyphosate is widely used in arecanut plantations for effective management of the diverse annual and perennial weeds that compete with palms for nutrients, water and light. Glyphosate [N- (phosphonomethyl) glycine] is commonly known as Roundup and is marketed under various trade names, including Glycel, Touchdown, Rodeo and Glyfos. It is a systemic, non-selective, broad-spectrum and post emergent herbicide. Its commercial success as a highly effective weed killer has stimulated extensive research on its mode of action, environmental behavior and soil persistence (Krzysko-Lupicka and Orlik, 1997; Forlani *et al.*, 1999; Jonge and Jonge, 1999). Glyphosate controls plants by inhibiting 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) in the shikimate pathway, blocking the biosynthesis of the aromatic amino acids phenylalanine, tyrosine and tryptophan, which leads to shikimic acid accumulation, cessation of protein synthesis and eventual plant death (Steinrucken and Amrhein, 1980; Duke and Powles, 2008). After foliar uptake, it is readily translocated through the phloem to meristematic tissues. In soil, glyphosate strongly adsorbs to clay minerals, iron and aluminum oxides and organic matter through hydrogen bonding and anion exchange, which restricts leaching but influences its availability and persistence period in soil (Aubin and Smith, 1992; Haney *et al.*, 2000; Veiga *et al.*, 2001). Reported soil half-lives typically range from a few days to two or three months, generally less than one growing season, although isolated studies have documented persistence for hundreds of days and phytotoxic activity lasting more than 19 weeks after application (Heinonen-Tanski, 1989). Microbial degradation to amino methyl phosphonic acid (AMPA) is the principal pathway of dissipation, with field studies reporting half-life values commonly between 9 and 38 days depending on soil texture, pH, organic matter content and climatic conditions (Giesy *et al.*, 2000).

Soil microorganisms play a crucial role in maintaining soil fertility through organic matter decomposition, nutrient mineralization, enzyme production and overall soil ecosystem functioning. Bacteria, fungi and actinomycetes are particularly important in plantation soils, where continuous litter input and high moisture create favourable conditions for microbial activity. Any disturbance to microbial communities can significantly affect nutrient cycling, soil structure and crop productivity. Glyphosate can influence soil microorganisms both directly and indirectly. Although it targets the shikimate pathway in plants, many soil microorganisms also possess this pathway, making them potentially susceptible to glyphosate exposure (Busse *et al.*, 2001). Some microorganisms may utilize glyphosate as a carbon or phosphorus source, while others may be inhibited due to toxic effects or changes in soil chemical properties (Zaller *et al.*, 2014).

Despite the widespread use of glyphosate in arecanut plantations, systematic information on its impact on soil microbial counts under arecanut-based cropping systems is limited. Understanding these effects is essential for evaluating the sustainability of chemical weed management practices and for developing integrated weed management strategies that protect soil biological health. Therefore, the present investigation was undertaken to study the effect of glyphosate application on bacterial, fungal and actinomycetes populations in soils under arecanut plantation.

Material and Methods

A field experiment was conducted during 2023-24 at Farmer's field, Abbalagere village, Shivamogga taluk to assess the residue levels of glyphosate (herbicide) in soil under arecanut plantation.

Details of the experiment

Location	Farmer's field, Abbalagere village, Shivamogga taluk
Crop	Arecanut
Spacing	2.7 m × 2.7 m
Plot size	8.1 m × 5.4 m
Design	RCBD
Treatment:	5
Replication:	4

Treatment details

T_1- Absolute control (Water spray)

T_2- Glyphosate (41% SL) @ 1.23 (3 L ha^{-1}) kg a.i. ha^{-1} (Recommended dose)

T_3- Glyphosate (41% SL) @ 2.05 (5 L ha^{-1}) kg a.i. ha^{-1}

T_4- Glyphosate (41% SL) @ 2.87 (7 L ha^{-1}) kg a.i. ha^{-1}

T_5- Glyphosate (41% SL) @ 3.69 (9 L ha^{-1}) kg a.i. ha^{-1}

Note: Glyphosate was applied as foliar spray for three times at two months interval.

The soil sample was collected from each treatment at different interval *i.e.*, 0th, 30th and 60th day from the arecanut plantation and were assessed for microbial counts (bacteria, fungi and actinomycetes).

Total microbial count (bacteria, fungi and actinomycetes) in soils

Enumeration of living microorganisms in the soil was carried out through dilution and plate count technique. Ten gram of soil suspension was taken and made up to 100 ml water for a 10^{-1} dilution. From this suspension, one ml was dispensed in nine ml water to get a 10^{-2} dilution. The same process was followed and diluted up to 10^{-6}. From the 10^{-6} dilutions, one ml was plated in a sterile petri plate for the enumeration of bacteria. Similarly, one ml of the 10^{-4} and 10^{-3} dilutions were used for fungi and actinomycetes counts, respectively. The plates with added 15 ml of the appropriate medium were then rotated in a clockwise and anticlockwise direction and left to solidify; afterwards, the plates were incubated upside down at room temperature. Following the incubation period, colonies were counted, assuming that every viable cell would produce a colony. Lastly, the number of colonies (CFU) in one g of soil was calculated using the following formula described by Skinner *et al.*, (1952).

$$\text{No. of colonies g}^{-1}\text{ soil (CFU g}^{-1}) = \frac{\text{No. of microbial colonies} \times \text{Dilution factor}}{\text{Suspension taken for dilution }(ml) \times \text{ weight of soil (g)}}$$

Statistical analysis

The analysis and interpretation of the data was done using Fisher's method of analysis of variance (ANOVA) as given by Gomez and Gomez (1984). Significance between the treatments was tested by F test. Whereas, difference between the treatments mean were tested by critical difference (CD) at 5 % level of significance.

Result and Discussion

Effect of different doses and frequency of glyphosate application soil bacterial population under arecanut plantation

The data in Table 1 depicted the variations in soil bacterial population in response to increasing glyphosate concentrations after the first, second and third spray.

The influence of repeated glyphosate applications on soil bacterial populations revealed a consistent pattern of initial inhibition followed by partial recovery over time. At the initial application, bacterial populations ranged from 36.11 ×10^{6} cfu g^{-1} soil (1.23 kg a.i. ha^{-1}) to 40.90 × 10^{6} cfu g^{-1} soil (control) at 0 day after spraying (DAS). The differences were statistically no significant, indicating that glyphosate exerted no immediate toxic effect on bacterial abundance. However, by 30 DAS, a dose dependent decline was observed, with the highest bacterial population recorded in 1.23 kg a.i. ha^{-1} (31.22 × 10^{6} cfu g^{-1} soil) and the lowest in 3.69 kg a.i. ha^{-1} (19.90 × 10^{6} cfu g^{-1} soil). A moderate resurgence occurred by 60 DAS, where bacterial counts increased across all treatments, though they remained significantly lower than the control (41.87 × 10^{6} cfu g^{-1} soil). Among treated plots, 1.23 kg a.i. ha^{-1} (33.78 × 10^{6} cfu g^{-1} soil) maintained the highest recovery, while 2.87 kg a.i. ha^{-1} (22.15 × 10^{6} cfu g^{-1} soil) and 3.69 kg a.i. ha^{-1} (20.90 × 10^{6} cfu g^{-1} soil) exhibited on par and persistent suppression, highlighting the inhibitory effect

Table 1: Effect of different doses and frequency of glyphosate application on soil bacterial population under arecanut plantation.

Treatments	Soil bacterial population (× 10^{6} cfu g^{-1} soil)								
	First spray			Second spray			Third spray		
	0th day	30 DAS	60 DAS	0th day	30 DAS	60 DAS	0th day	30 DAS	60 DAS
T_1- Control (Water spray)	40.90	41.30	41.87	42.00	42.65	43.97	44.60	45.90	47.00
T_2- Glyphosate (41% SL) @ 1.23 kg a.i. ha^{-1}	38.27	31.22	33.78	34.00	29.18	30.22	31.06	24.20	25.16
T_3- Glyphosate (41% SL) @ 2.05 kg a.i. ha^{-1}	38.02	27.61	28.03	28.75	21.35	21.98	22.59	16.97	17.75
T_4- Glyphosate (41% SL) @ 2.87 kg a.i. ha^{-1}	37.39	21.53	22.15	22.21	17.08	17.85	18.11	12.68	13.00
T_5- Glyphosate (41% SL) @ 3.69 kg a.i. ha^{-1}	36.11	19.90	20.90	21.36	16.13	16.92	17.78	11.00	11.38
S.Em ±	1.43	0.99	1.36	1.27	1.24	0.81	0.95	0.84	0.71
CD at 5%	NS	3.04	4.20	3.90	3.81	2.48	2.92	2.58	2.19

Note: DAS-Days After Spray; NS- Non Significant of higher glyphosate concentrations.

Following the second glyphosate spray, the inhibitory trend became more evident. At 0 DAS, bacterial populations in the control (42.00 × 10^6 cfu g^{-1} soil) were significantly higher than those in glyphosate treated plots, with 1.23 kg a.i. ha^{-1} (34.00 × 10^6 cfu g^{-1} soil) recorded the maximum among the glyphosate treatments and 3.69 kg a.i. ha^{-1} (21.36 × 10^6 cfu g^{-1} soil) the minimum. After 30 days, further reduction was evident, particularly at higher doses. The bacterial count in 1.23 kg a.i. ha^{-1} (29.18 × 10^6 cfu g^{-1} soil) remained relatively higher than the other treatments, whereas 2.87 kg a.i. ha^{-1} (17.08 × 10^6 cfu g^{-1} soil) and 3.69 kg a.i. ha^{-1} (16.13 × 10^6 cfu g^{-1} soil) recorded the lowest and on par values. A slight recovery was again observed by 60 DAS, though populations in treated plots remained below the control (43.97 × 10^6 cfu g^{-1} soil). Among treatments, 1.23 kg a.i. ha^{-1} (30.22 × 10^6 cfu g^{-1} soil) showed the highest bacterial count, while 2.87 kg a.i. ha^{-1} (17.85 × 10^6 cfu g^{-1} soil) and 3.69 kg a.i. ha^{-1} (16.92 × 10^6 cfu g^{-1} soil) continued to exhibit marked suppression, suggesting cumulative stress from repeated glyphosate exposure.

During the third spray, the impact of glyphosate became even more pronounced. At 0 DAS, bacterial counts ranged from 17.78 × 10^6 cfu g^{-1} soil in T_5 (3.69kg a.i. ha^{-1}) to 44.60 × 10^6 cfu g^{-1} soil in control, with T_2 (1.23 kg a.i. ha^{-1}) maintaining a relatively higher count (31.06 × 10^6 cfu g^{-1} soil) among treated plots. By 30 DAS, bacterial numbers declined drastically in all treated soils compared to control (45.90 × 10^6 cfu g^{-1} soil). Application of glyphosate at 1.23 kg a.i. ha^{-1} (T_2) again showed comparatively better survival (24.20 × 10^6 cfu g^{-1} soil), while T_4 (2.87 kg a.i. ha^{-1}) and T_5 (3.69 kg a.i. ha^{-1}) recorded the lowest populations with 12.68 × 10^6 and 11.00 × 10^6 cfu g^{-1} soil, respectively significantly below the control. Even at 60 DAS, recovery was minimal, with the control exhibiting 47.00 × 10^6 cfu g^{-1} soil, whereas T_2 (25.16 × 10^6 cfu g^{-1}soil) remained the most resilient among the treated plots. The lowest counts were recorded in T_5 (11.38 × 10^6 cfu g^{-1} soil) which is on par with T_4 (13.00 × 10^6 cfu g^{-1} soil) and confirming sustained suppression of bacterial activity at higher glyphosate concentrations and under repeated exposure.

Effect of different doses and frequency of glyphosate application on soil fungal population under arecanut plantation

The data presented in Table 2 illustrated the effect of different glyphosate levels (41% SL) on soil fungal population at 0, 30 and 60 days after each spray.

During the first glyphosate application, fungal populations showed only slight and statistically non significant variation among the treatments at 0 days after spraying (DAS). The control maintained the highest population (30.04 × 10^4 cfu g^{-1} soil). But, soils treated with glyphosate (41% SL) @ 1.23 kg a.i. ha^{-1} recorded 29.15 × 10^4 cfu g^{-1} soil, 2.05 kg a.i. ha^{-1} with 28.83 × 10^4 cfu g^{-1} soil, 2.87 kg a.i. ha^{-1} with 28.22 × 10^4 cfu g^{-1} soil and 3.69 kg a.i. ha^{-1} with 27.05 × 10^4 cfu g^{-1} soil. These results indicated minimal immediate impact of glyphosate on the soil fungal community. By 30 DAS, a significant decline in fungal populations was observed with increasing glyphosate concentration. The control recorded the highest population (32.40 × 10^4 cfu g^{-1} soil), while populations decreased progressively in soils treated with 1.23, 2.05, 2.87 and 3.69 kg a.i. ha^{-1}, registering 21.00, 18.75, 13.67 and 12.93 × 10^4 cfu g^{-1} soil, respectively. The reduction was most evident at higher doses, where 2.87 and 3.69 kg a.i. ha^{-1} were statistically at par. At 60 DAS, a slight recovery was evident in all treatments, though fungal populations remained lower than the control (33.63 × 10^4 cfu g^{-1} soil). Among the treated soils, 1.23 kg a.i. ha^{-1} exhibited the highest population (22.85 × 10^4 cfu g^{-1} soil), followed by 2.05 kg a.i. ha^{-1} (19.35 × 10^4 cfu g^{-1} soil), while the higher doses (2.87 and 3.69 kg a.i. ha^{-1}) recorded lower but comparable counts (14.95 and 13.08 × 10^4 cfu g^{-1} soil).

Table 2: Effect of different doses and frequency of glyphosate application on soil fungal population under arecanut plantation.

Treatments	Soil fungal population (× 10^4 cfu g^{-1} soil)								
	First spray			Second spray			Third spray		
	0th day	30 DAS	60 DAS	0th day	30 DAS	60 DAS	0th day	30 DAS	60 DAS
T_1- Control (Water spray)	30.04	32.40	33.63	33.97	34.66	35.11	35.82	36.38	37.20
T_2- Glyphosate (41% SL) @ 1.23 kg a.i. ha^{-1}	29.15	21.00	22.85	23.06	16.26	17.22	18.18	9.31	10.88
T_3- Glyphosate (41% SL) @ 2.05 kg a.i. ha^{-1}	28.83	18.75	19.35	19.52	11.77	12.63	13.53	7.02	7.81
T_4- Glyphosate (41% SL) @ 2.87 kg a.i. ha^{-1}	28.22	13.67	14.95	15.16	8.93	10.28	11.00	5.34	5.99
T_5- Glyphosate (41% SL) @ 3.69 kg a.i. ha^{-1}	27.05	12.93	13.08	13.42	8.12	9.50	10.23	4.19	4.82
S.Em ±	1.28	0.65	0.81	0.95	0.69	0.64	0.68	0.50	0.57
CD at 5%	NS	2.01	2.49	2.92	2.14	1.98	2.10	1.55	1.74
Note: DAS-Days After Spray; NS- Non Significant									

At the beginning of the second glyphosate application (0 DAS), fungal populations had already declined compared to the first spray, suggesting a residual inhibitory effect of the herbicide. The control recorded the highest population (33.97 × 10^4 cfu g^{-1} soil), while the lowest was observed due to glyphosate application at 3.69 kg a.i. ha^{-1} (13.42 ×10^4 cfu g^{-1} soil). Among treated soils, 1.23 kg a.i. ha^{-1} maintained relatively higher populations (23.06 × 10^4 cfu g^{-1} soil), followed by 2.05 kg a.i. ha^{-1} (19.52× 10^4 cfu g^{-1} soil), whereas 2.87 and 3.69 kg a.i. ha^{-1} showed significantly lower values (15.16 and 13.42 × 10^4 cfu g^{-1} soil), which were statistically similar. By 30 DAS, the inhibitory effect became more pronounced. The control exhibited the highest population (34.66 × 10^4 cfu g^{-1} soil), while glyphosate treated soils recorded 16.26, 11.77, 8.93 and 8.12 × 10^4 cfu g^{-1} soil for 1.23, 2.05, 2.87 and 3.69 kg a.i. ha^{-1}, respectively, showing a dose dependent decline. At 60 DAS, a marginal recovery in fungal populations was observed, but the values remained significantly lower than in the control (35.11 × 10^4 cfu g^{-1} soil). Among the treated soils, 1.23 kg a.i. ha^{-1} recorded the highest fungal count (17.22 × 10^4 cfu g^{-1} soil), followed by 2.05 kg a.i. ha^{-1} (12.63 × 10^4 cfu g^{-1} soil), while 2.87 and 3.69 kg a.i. ha^{-1} showed similar and lowest populations (10.28 and 9.50 × 10^4 cfu g^{-1} soil).

Following the third glyphosate application, fungal populations exhibited a further decline compared to earlier sprays, demonstrating the cumulative suppressive effect of repeated herbicide exposure. At 0 DAS, the control recorded the highest fungal population (35.82 × 10^4 cfu g^{-1} soil). Among the treated plots, the population was highest at 1.23 kg a.i. ha^{-1} (18.18 × 10^4 cfu g^{-1} soil), followed by 2.05 kg a.i. ha^{-1} (13.53× 10^4 cfu g^{-1} soil), whereas 2.87 and 3.69 kg a.i. ha^{-1} recorded the lowest but comparable counts (11.00 and 10.23 × 10^4 cfu g^{-1} soil). By 30 DAS, the fungal population declined sharply in all glyphosate treated soils, while the control maintained a higher count (36.38 × 10^4 cfu g^{-1} soil). Populations at 1.23, 2.05, 2.87 and 3.69 kg a.i. ha^{-1} were 9.31, 7.02, 5.34 and 4.19× 10^4 cfu g^{-1} soil, respectively, indicating strong inhibitory effects at higher concentrations. At 60 DAS, a minor recovery was evident across all treatments, although the values remained considerably below the control (37.20 × 10^4 cfu g^{-1} soil). The treatment with 1.23 kg a.i. ha^{-1} recorded the highest fungal count (10.88 × 10^4 cfu g^{-1}soil), followed by 2.05 kg a.i. ha^{-1} (7.81 × 10^4 cfu g^{-1}soil), whereas the lowest and statistically comparable counts were observed at 2.87 and 3.69 kg a.i. ha^{-1} (5.99 and 4.82 × 10^4 cfu g^{-1} soil).

Effect of different doses and frequency of glyphosate application on soil actinomycetes population under arecanut plantation

The data presented in Table 3 depicted the effect of different glyphosate levels (41% SL) on soil actinomycetes population at 0, 30 and 60 days after each spray.

At 0 days after spraying (DAS) of glyphosate, the differences in actinomycetes populations among treatments were non significant, with values ranging from 14.01 × 10^5 cfu g^{-1} soil (3.69 kg a.i. ha^{-1}) to 16.22 × 10^5 cfu g^{-1} soil (control) in first spray. By 30 DAS, a noticeable decline in actinomycetes populations was observed with increasing glyphosate concentrations. Among the treated plots, the population was significantly higher in 1.23 kg a.i. ha^{-1} (9.23 × 10^5 cfu g^{-1} soil), followed by 2.05 kg a.i. ha^{-1} (7.02 × 10^5 cfu g^{-1} soil), 2.87 kg a.i. ha^{-1} (6.10 × 10^5 cfu g^{-1} soil) and 3.69 kg a.i. ha^{-1} (5.30 × 10^5 cfu g^{-1} soil), however all treated plots recorded significantly lower populations compared to the control (16.89 × 10^5 cfu g^{-1} soil). At 60 DAS, a slight recovery in actinomycetes populations was evident across all treatments. The highest population among the treated plots was recorded in 1.23 kg a.i. ha^{-1} (10.80 × 10^5 cfu g^{-1} soil), while the lowest

Table 3: Effect of different doses and frequency of glyphosate application on soil actinomycetes population under arecanut plantation.

Treatments	Soil actinomycetes population (× 10^5cfu g^{-1} soil)								
	First spray			Second spray			Third spray		
	0th day	30 DAS	60 DAS	0th day	30 DAS	60 DAS	0th day	30 DAS	60 DAS
T_1- Control (Water spray)	16.22	16.89	17.02	17.79	18.12	18.25	18.99	19.03	19.50
T_2- Glyphosate (41% SL) @ 1.23 kg a.i. ha^{-1}	15.95	9.23	10.80	11.80	8.86	9.71	10.44	6.92	7.30
T_3- Glyphosate (41% SL) @ 2.05 kg a.i. ha^{-1}	15.63	7.02	8.00	8.93	5.24	6.84	7.68	4.83	5.21
T_4- Glyphosate (41% SL) @ 2.87 kg a.i. ha^{-1}	14.78	6.10	7.12	7.20	3.18	4.75	5.52	2.16	3.32
T_5- Glyphosate (41% SL) @ 3.69 kg a.i. ha^{-1}	14.01	5.30	6.63	6.70	2.76	3.13	4.02	1.39	1.90
S.Em ±	0.73	0.26	0.28	0.26	0.30	0.59	0.53	0.29	0.48
CD at 5%	NS	0.82	0.85	0.79	0.93	1.82	1.63	0.89	1.49
Note: DAS-Days After Spray; NS- Non Significant									

populations were observed in 2.87 kg a.i. ha^{-1} (7.12×10^5 cfu g^{-1} soil) and 3.69 kg a.i. ha^{-1} (6.63×10^5 cfu g^{-1} soil), which were statistically on par.

In second spray, the actinomycetes population had declined compared to the first spray, at 0 days after spraying (DAS), indicating a residual inhibitory effect of glyphosate. The control recorded the highest population (17.79×10^5 cfu g^{-1} soil), while the glyphosate treated plots showed progressively lower counts with increasing herbicide concentration *i.e.*, 11.80, 8.93, 7.20 and 6.70×10^5 cfu g^{-1} soil in treatments T_2 (1.23 kg a.i. ha^{-1}), T_3 (2.05 kg a.i. ha^{-1}), T_4 (2.87 kg a.i. ha^{-1}) and T_5 (3.69 kg a.i. ha^{-1}), respectively. By 30 DAS, the suppressive effect became more pronounced, with actinomycetes populations further decreasing in the treated soils. The control maintained significantly higher counts (18.12×10^5 cfu g^{-1} soil), whereas populations in T_2, T_3, T_4 and T_5 declined to 8.86, 5.24, 3.18 and 2.76×10^5 cfu g^{-1} soil, respectively. At 60 DAS, a marginal recovery was observed across all treatments; however populations remained significantly lower than the control (18.25×10^5 cfu g^{-1} soil). Among the treated plots, T_2 (9.71×10^5 cfu g^{-1} soil) recorded the highest population, followed by T_3 (6.84 10^5 cfu g^{-1} soil), while T_4 (4.75×10^5 cfu g^{-1} soil) and T_5 (3.13×10^5 cfu g^{-1} soil) were statistically comparable.

Following the third spray, the inhibitory effect of glyphosate on soil actinomycetes populations became more pronounced. At 0 DAS, populations ranged from 4.02×10^5 cfu g^{-1} soil (3.69 kg a.i. ha^{-1}) to 18.99×10^5 cfu g^{-1} soil (control). Among treated plots, 1.23 kg a.i. ha^{-1} (10.44×10^5 cfu g^{-1} soil) recorded the significantly highest population, followed by 2.05 kg a.i. ha^{-1} (7.68×10^5 cfu g^{-1} soil), while 2.87 kg a.i. ha^{-1} (5.52×10^5 cfu g^{-1} soil) and 3.69 kg a.i. ha^{-1} (4.02×10^5 cfu g^{-1} soil) were statistically similar. By 30 DAS, a sharp reduction was evident, with 6.92×10^5, 4.83×10^5, 2.16×10^5 and 1.39×10^5 cfu g^{-1} soil in T_2 (1.23 kg a.i. ha^{-1}), T_3 (2.05 kg a.i. ha^{-1}), T_4 (2.87 kg a.i. ha^{-1}) and T_5 (3.69 kg a.i. ha^{-1}), whereas the control maintained a steady population (19.03×10^5 cfu g^{-1}soil). At 60 DAS, only a marginal recovery occurred; T_2 (7.30×10^5 cfu g^{-1} soil) recorded the highest population among treated plots, while T_4 (3.32×10^5 cfu g^{-1} soil) and T_4 (1.90×10^5 cfu g^{-1} soil) were statistically on par and remained far below the control (19.50×10^5 cfu g^{-1} soil).

The application of glyphosate herbicide exerted a significant influence on soil microbial populations, including bacteria, fungi and actinomycetes, showing consistent trends across all three sprays. The microbial population was highest in the untreated control (T_1) throughout the experimental period, whereas a progressive reduction was evident with increasing glyphosate doses (T_2-T_5). This decline was particularly pronounced at 30 days after spraying (DAS), followed by partial recovery at 60 DAS, indicating an initial inhibitory phase followed by gradual adaptation as the herbicide dissipated. The consistently higher microbial counts in the control plots can be attributed to the absence of chemical stress, allowing normal metabolic, enzymatic and nutrient cycling processes to continue uninterrupted. Nannipieri *et al.*, (2003) emphasized that chemical disturbances often reduce microbial biomass and respiration, whereas Van Bruggen *et al.*, (2018) reported that untreated soils maintain stable and functionally redundant microbial networks capable of sustaining nutrient cycling.

At 0 DAS, microbial populations did not differ significantly among treatments, suggesting that glyphosate required time to interact with soil components and microbial cells before exerting measurable inhibitory effects. However, by 30 DAS, significant declines in bacterial, fungal and actinomycetes populations were recorded across all glyphosate treatments, particularly at higher concentrations (T_4 and T_5). Araujo *et al.*, (2003) observed similar reductions in coffee soils, where glyphosate applications caused marked decreases in fungi and actinomycetes, demonstrating the sensitivity of these groups to herbicide exposure. The inhibition observed in the present study corresponds to the period when glyphosate remains most bioavailable and metabolically active in the soil solution. Glyphosate's primary toxic mechanism is the inhibition of the enzyme 5-enolpyruvylshikimate-3-phosphate synthase, thereby blocking the synthesis of aromatic amino acids such as phenylalanine, tyrosine and tryptophan essential for microbial protein formation (Zabaloy *et al.*, 2012). As reported by Busse *et al.*, (2001) and Druille *et al.*, (2013), this disruption results in a temporary suppression of soil microbial biomass and enzymatic activity. Similarly, Naik

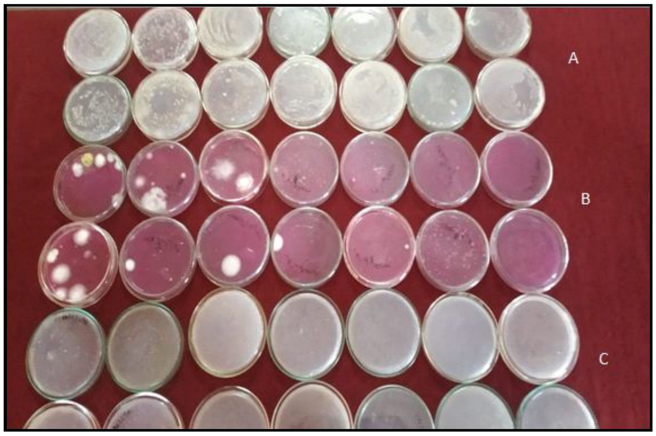

Fig. 1: Representative colonies of bacteria (A), fungi (B) and actinomycetes (C) isolated from glyphosate treated soil samples.

et al., (2025) found a decline in total microbial counts in arecanut soils following successive glyphosate sprays, confirming that higher doses intensify inhibitory effects.

Glyphosate's chelating property further aggravates microbial stress by immobilizing micronutrients such as Fe^{2+}, Zn^{2+}, Mn^{2+} and Cu^{2+}, which are essential cofactors for microbial enzymatic systems (Eker *et al.,* 2006; Zobiole *et al.,* 2011). This phenomenon reduces nutrient bioavailability and disrupts microbial metabolism, as also demonstrated by Mertens *et al.,* (2018), who showed that glyphosate-metal complexes can inhibit soil enzyme synthesis. In agreement, Sebiomo *et al.,* (2010) observed significant decreases in microbial populations and dehydrogenase activity in soils treated with herbicides, confirming dose dependent suppression. Similar findings were reported by Ratcliff *et al.,* (2006) in corn-soybean rotation soils, Nguyen *et al.,* (2016) in rice-vegetable cropping soils and Tang *et al.,* (2020) in paddy soils, who noted significant, dose dependent decreases in soil microbial abundance and enzyme activity under glyphosate application. Bo *et al.,* (2011) reported that repeated glyphosate applications altered microbial community structure and lowered microbial carbon use efficiency, further supporting the present findings. Comparable results by Al-Ani *et al.,* (2019) indicated that glyphosate induced reductions in bacterial and fungal colonies were closely related to micronutrient chelation and decreased organic carbon availability.

Among the microbial groups, fungi and actinomycetes were more adversely affected than bacteria. Araujo *et al.,* (2003) and Druille *et al.,* (2013) both noted that fungi are particularly sensitive to oxidative stress and carbon limitation under herbicide exposure, which impairs spore germination and hyphal elongation. Actinomycetes, being slow growing decomposers of complex organic matter, are highly responsive to changes in soil pH and nutrient status (Sannino and Gianfreda, 2001). Similar findings were reported by Zain *et al.,* (2013) in oil palm soils and by Shitha *et al.,* (2017) in tea growing regions, where glyphosate applications significantly decreased fungal and actinomycetes populations. Kumar *et al.,* (2017) observed that these groups were slower to recover even after herbicide dissipation, suggesting lasting functional effects on organic matter decomposition. Bashir *et al.,* (2018) also found that glyphosate treatments reduced actinomycete biomass and enzymatic potential, implying that chronic exposure can alter microbial decomposition capacity.

In contrast, bacterial populations exhibited a comparatively faster recovery, especially by 60 DAS, likely due to the proliferation of glyphosate tolerant or degrading strains. Dick and Quinn (1995) first demonstrated that species of *Pseudomonas* and *Arthrobacter* possess the enzyme glyphosate oxidoreductase, enabling them to degrade glyphosate into aminomethylphosphonic acid. Kryuchkova *et al.,* (2014) further confirmed that these bacteria can use glyphosate as a source of phosphorus and carbon, facilitating recovery and soil detoxification. Such adaptation was also reported by Chandupatla *et al.,* (2021), who observed increased abundance of *Bacillus* spp. in herbicide exposed soils, indicating selection of tolerant strains. The present study's recovery trend at 60 DAS aligns with the findings of Gimsing *et al.,* (2004) and Okada *et al.,* (2016), who demonstrated that glyphosate mineralization and adsorption to soil colloids reduce its bioavailability and toxicity. Accinelli *et al.,* (2002) also noted that microbial respiration gradually normalized as glyphosate residues dissipated, highlighting microbial resilience and enzymatic adaptability.

Successive glyphosate applications, however, caused cumulative suppression, with each subsequent spray amplifying microbial inhibition and delaying recovery. Zaller *et al.,* (2014) and Helander *et al.,* (2018) observed similar cumulative effects in European agricultural soils, where repeated applications led to progressive declines in soil microbial diversity and delayed restoration of metabolic activity. Vazquez *et al.,* (2020) reported that continuous glyphosate exposure not only reduced total microbial biomass but also altered fungal community composition, particularly suppressing arbuscular mycorrhizal fungi. Abdullahi *et al.,* (2022) further confirmed that prolonged herbicide use in tropical soils results in long term shifts in bacterial community structure and reduced enzyme mediated nutrient turnover. Collectively, these findings suggest that chronic glyphosate stress can impair microbial balance, reduce soil biological fertility and compromise nutrient cycling.

Overall, the present study confirms that glyphosate application to soil transiently suppresses bacterial, fungal and actinomycetes populations in soil, with the extent of inhibition depending on herbicide dose and frequency. While bacterial populations show partial resilience due to metabolic adaptability, fungal and actinomycete communities are more vulnerable to glyphosate induced stress. Comparable conclusions were drawn by Jayamadhuri and Rangaswamy (2005), who linked reductions in microbial biomass to decreased enzyme activity and by Ratcliff *et al.,* (2006) and Nguyen *et al.,* (2016), who emphasized that soil microbial recovery is primarily driven by glyphosate degradation and diminishing bioavailability. Thus, although the inhibition observed up to 30 DAS and recovery by 60 DAS represent a transient ecological disturbance, repeated applications could cumulatively impair soil health. Hence, integrating glyphosate within an ecologically balanced weed management framework is crucial to sustain microbial diversity and soil ecosystem functionality.

Conclusion

Repeated glyphosate application significantly affected soil microbial populations in a dose and frequency dependent manner under arecanut plantation. Bacterial, fungal, and actinomycetes populations showed no immediate adverse effects at 0 DAS, but a pronounced decline was evident by 30 DAS, particularly at higher doses (2.87 and 3.69 kg a.i. ha^{-1}). Although partial recovery was observed by 60 DAS, microbial populations remained consistently lower than the control and recovery diminished with successive sprays. Fungi and actinomycetes exhibited greater sensitivity to glyphosate than bacteria. The lowest dose (1.23 kg a.i. ha^{-1}) supported relatively higher microbial abundance and faster recovery. These findings emphasize the importance of judicious glyphosate use to maintain soil microbial balance and long term soil health.

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