



RESEARCH COMMUNICATION

# Response of *Bradyrhizobium* and soil microbial communities to premix herbicides in black gram

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## Abstract

Broad-spectrum premix herbicides, combining active ingredients with different modes of action are effective at lower doses. However, these may pose a significant risk of non-target effects to soil microorganisms beneficial for agricultural production. We studied the response of black gram specific *Bradyrhizobium* strain BMBS 47 and rhizospheric microbial communities to 2 premix herbicides viz., pendimethalin (30 %) + imazethapyr (2 %) (Penza 32 % emulsifiable concentrate (EC)) and propaquizafop (2.5 %) + imazethapyr (3.75 %) (Shaked 6.25 % microemulsion (ME)). Laboratory sensitivity assays using the disc diffusion method revealed no inhibitory zones against *Bradyrhizobium* across a wider concentration, which confirm complete tolerance to both the formulations. Field experiments further revealed that soil microbial population responded variably to weed management practices. Rhizobium count was significantly higher in pendimethalin + imazethapyr treated plots due to favourable rhizospheric condition provided by early stage weed suppression, whereas lower counts occurred under sequential herbicide applications or hand weeding. Soils treated with pendimethalin + imazethapyr application recorded a higher fungal population ( $6.20 \log_{10}$  CFU  $g^{-1}$ ) which was comparable to weedy check ( $5.56 \log_{10}$  CFU  $g^{-1}$ ) at 30 days after sowing (DAS). Population of phosphorus-solubilising bacteria remained unaffected, indicating that their population was unaltered under sufficient soil phosphorus levels. For free-living nitrogen fixers and actinomycetes, there appeared to be time and treatment-specific variations, whereas fungal population was unaffected or enhanced under pendimethalin + imazethapyr, confirming the absence of harmful effects. The weedy check soil had higher microbial abundance at 30 DAS, could be due to diverse weed root exudates and reduced soil disturbance. The study confirmed the suitability of the premix herbicide, pendimethalin + imazethapyr, as component of integrated weed management in black gram without negative impact on the short-term survival of beneficial soil microbes.

**Keywords:** *Bradyrhizobium*; microbial diversity; nitrogen fixers; premix herbicides; soil health

## Introduction

The development of premix herbicide formulations has provided newer options for effective and broad-spectrum weed control in agricultural systems. These formulations, apart from having two or more active ingredients with different modes of action, allow management of diverse weed flora at comparatively lower application rates. The use of premix combinations either as pre- or post-emergence extends the period of weed control, thereby reducing crop-weed competition during critical growth stages minimising the risk of yield losses. Another advantage is that premix herbicides can help to delay the development of herbicide resistant weeds since they act through multiple biochemical pathways in weeds.

The efficacy of herbicides is not always the same across environments and may fluctuate depending on several climatic and edaphic factors, including temperature, soil type, organic matter content and moisture availability. Moreover, apart from their in-weed control, the herbicide residues deposited on the soil or incorporated into the rhizosphere may interact with non-target soil biota. Understanding the impact of herbicide residues on soil microbial

communities and ecosystem functions is critical for advancing sustainable agriculture. Such interactions can influence the abundance and diversity of beneficial microbial communities, alter enzyme activities and affect key soil functions related to nutrient cycling. Soil microorganisms are among the earliest living components affected when toxic compounds enter the soil environment, either through direct contact or secondary interactions. Herbicides can enter bacterial cells, interfere with metabolic processes and may lead to the elimination of susceptible members of the soil microbial community (1).

Certain herbicide residues can, in the short term can reduce phosphorus uptake, decrease carbon mineralisation and inhibit nitrogen fixation, thereby altering overall soil functions (2). The disruption of beneficial rhizospheric microflora such as symbiotic nitrogen-fixing bacteria *Bradyrhizobium* and other beneficial microbes can have significant effect on soil health, crop productivity and ecological balance. Pendimethalin and clethodim have been reported to impair nitrogen fixation in *Phaseolus vulgaris* L. and *Medicago sativa* L. by inhibiting root growth, altering root exudation and reducing rhizobial fitness (3). Therefore, understanding the

potential impact of premix herbicide residues on beneficial rhizospheric microorganisms is essential for developing integrated weed management strategies that maintain both crop productivity and soil microbial integrity. As the herbicide fraction on soil can influence the dynamics of soil microbial population and beneficial microorganisms like *Bradyrhizobium*, the present study was undertaken to study the effect of premix herbicides on beneficial microflora in black gram.

## Materials and Methods

### Media preparation

The *in vitro* sensitivity of the black gram-specific *Bradyrhizobium* strain BMBS 47 to two premix herbicides was assessed using laboratory assays, employing the disc diffusion method and carried out in accordance with standard protocols (4). The strain collected from the Department of Agricultural Microbiology, Tamil Nadu Agricultural University (TNAU), Coimbatore, was cultured on yeast extract mannitol agar (YEMA) medium, prepared with mannitol (10 g L<sup>-1</sup>), K<sub>2</sub>HPO<sub>4</sub> (0.5 g L<sup>-1</sup>), MgSO<sub>4</sub>·7H<sub>2</sub>O (0.2 g L<sup>-1</sup>), NaCl (0.1 g L<sup>-1</sup>), yeast extract (1.0 g L<sup>-1</sup>) and agar (15 g L<sup>-1</sup>). For preparation, 26.8 g of commercial YEMA powder (HiMedia Laboratories, Mumbai) was dissolved in 1000 mL distilled water, boiled for complete dissolution and sterilised by autoclaving at 15 lbs pressure (121 °C) for 15 min. The medium was cooled to 45–50 °C, mixed thoroughly and poured into sterile Petri plates under aseptic conditions. The experiment was laid out in a completely randomised design with 8 treatments (7 herbicide concentrations plus a control) having 3 replications.

### Preparation of stock solution of test herbicides

The herbicides tested were pendimethalin + imazethapyr and propaquizafop + imazethapyr. For the herbicide combination pendimethalin + imazethapyr (Penza 32 % EC), concentrations of 800, 1000, 1200, 1400, 1600, 1800 and 2000 ppm were tested, representing sub-field, field-equivalent and above field doses along with a control. A stock solution of 100000 ppm was prepared by dissolving 10 mL of the herbicide in 100 mL sterile water and the required concentrations were obtained by pipetting 800–2000 µL of the stock into 250 mL conical flasks and making up the volume to 100 mL with sterile water. For propaquizafop + imazethapyr (Shaked 6.25 % ME), the concentrations were 175, 200, 225, 250, 275, 300 and 325 ppm, representing sub-field, field-equivalent and above field doses along with a control. A stock solution of 20000 ppm was prepared by dissolving 2 mL of the herbicide in 100 mL sterile water and the required concentrations were prepared by pipetting 175 to 325 µL of the stock into 250 mL conical flasks and making up the volume to 100 mL with sterile water.

### Assessing *in vitro* sensitivity of *Bradyrhizobium*

The disc diffusion method was used for sensitivity testing (4). For this, solidified YEMA plates were swabbed evenly with a cell suspension from a four-day-old broth culture of *Bradyrhizobium* BMBS 47 using sterile cotton swabs. Sterile filter paper discs (6 mm diameter) dipped in the respective herbicide concentrations were placed at 3 equidistant positions on each plate, while discs dipped in sterile water served as control. The plates were sealed and incubated at room temperature (28 ± 2 °C) for 3 days.

### Isolation of microorganisms using general purpose and specific media

For assessing the microbial population dynamics, soil samples were

collected from the root zone of treatment plots before herbicide application and at 15 and 30 days after application of pre and post-emergence herbicides. The soil texture of the experimental site was red sandy loam, acidic in reaction and the herbicides were applied using a manually operated knapsack sprayer. The weed management treatments with premix herbicides were T<sub>1</sub> - pre-emergence (PE) application of pendimethalin + imazethapyr at 800 g ha<sup>-1</sup>; T<sub>2</sub> - T<sub>1</sub> followed by hand weeding (HW) at 30 days after sowing (DAS); T<sub>3</sub> - post-emergence (PoE) application of propaquizafop + imazethapyr at 125 g ha<sup>-1</sup>; T<sub>4</sub> - T<sub>3</sub> followed by HW at 40 DAS; T<sub>5</sub> - PE pendimethalin at 1 kg ha<sup>-1</sup> followed by HW at 30 DAS; T<sub>6</sub> - PE pendimethalin at 1 kg ha<sup>-1</sup> followed by imazethapyr at 50 g ha<sup>-1</sup>; T<sub>7</sub> - PE pendimethalin at 1 kg ha<sup>-1</sup> followed by propaquizafop at 100 g ha<sup>-1</sup>; T<sub>8</sub> - HW at 15 and 30 DAS and T<sub>9</sub> - weedy check. Different groups of soil microflora were isolated and enumerated using selective media, viz. nutrient agar for total bacteria (5), Martin's rose bengal agar for total fungi (6), Ken Knight's agar for actinomycetes (7), Jensen's medium for nitrogen-fixing bacteria (8) and Pikovskaya's agar for phosphate-solubilisers (9). Composite samples were prepared and 10 g of soil was transferred to 90 mL sterile water in a 250 mL conical flask and shaken at 200 rpm for 20 min to obtain a 10<sup>-1</sup> dilution. Serial dilutions up to 10<sup>-1</sup> were prepared under aseptic conditions in a laminar airflow cabinet. 1 mL aliquot from appropriate dilutions were transferred to sterile Petri dishes and respective media cooled to 45 °C was poured to the plates. Plates were gently swirled for uniform distribution, allowed to solidify and incubated in an inverted position at 28 ± 2 °C for 24–48 hr. Three replications were maintained for each dilution and microbial colonies were counted and expressed as colony-forming units (cfu) g<sup>-1</sup> soil (10).

## Results and Discussion

### *In vitro* sensitivity of *Bradyrhizobium* to herbicide mixtures

The *in vitro* assays showed that *Bradyrhizobium* growth was unaffected by either of the premix herbicides tested. For pendimethalin + imazethapyr, evaluated at concentrations from 800–2000 ppm, no inhibition zones were observed around the sterile discs placed on *Bradyrhizobium* lawns and positive growth occurred at all doses (Table 1). Similarly, propaquizafop + imazethapyr, tested at 175–325 ppm, produced no inhibition zones, indicating no detectable suppression of *Bradyrhizobium* growth (Fig. 1 and 2). Maintaining viable *Bradyrhizobium* population is essential for sustaining nitrogen input, soil fertility and crop productivity. The results demonstrated that both herbicide combinations, at the concentrations evaluated, exerted no inhibitory effect on the black gram-specific *Bradyrhizobium* strain under laboratory conditions. Studies showed that rhizobial strains are capable of degrading certain herbicides producing plant growth-promoting substances such as indole-3-acetic acid (IAA), which help plants tolerate herbicide-induced stress and enhance nitrogen fixation and soil fertility (11–13). Their tolerance suggests that pendimethalin + imazethapyr can be integrated into weed management programmes without compromising *Bradyrhizobium* survival, thereby supporting biological nitrogen fixation in a legume-based system with black gram as a component. Although laboratory results demonstrated compatibility, long-term field studies are necessary to validate these interactions under diverse soil and environmental conditions.

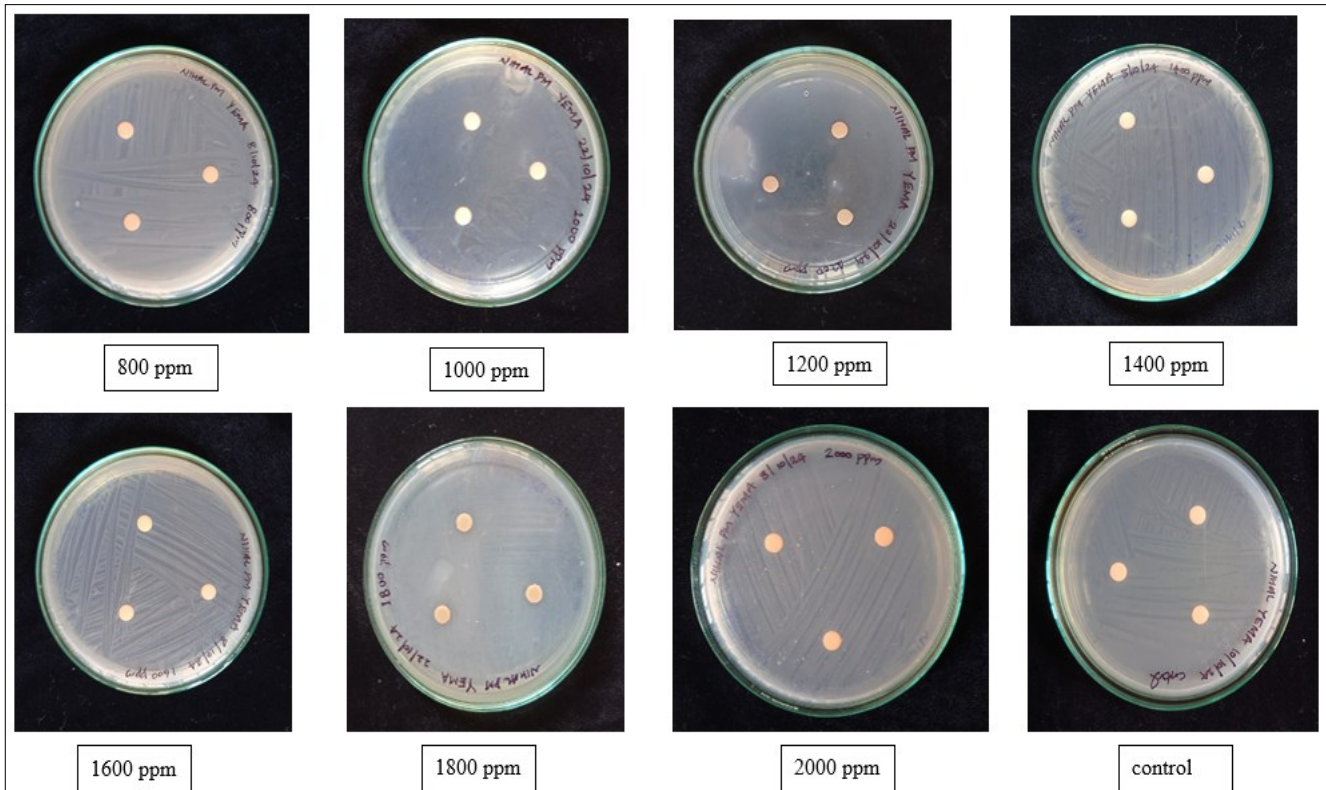
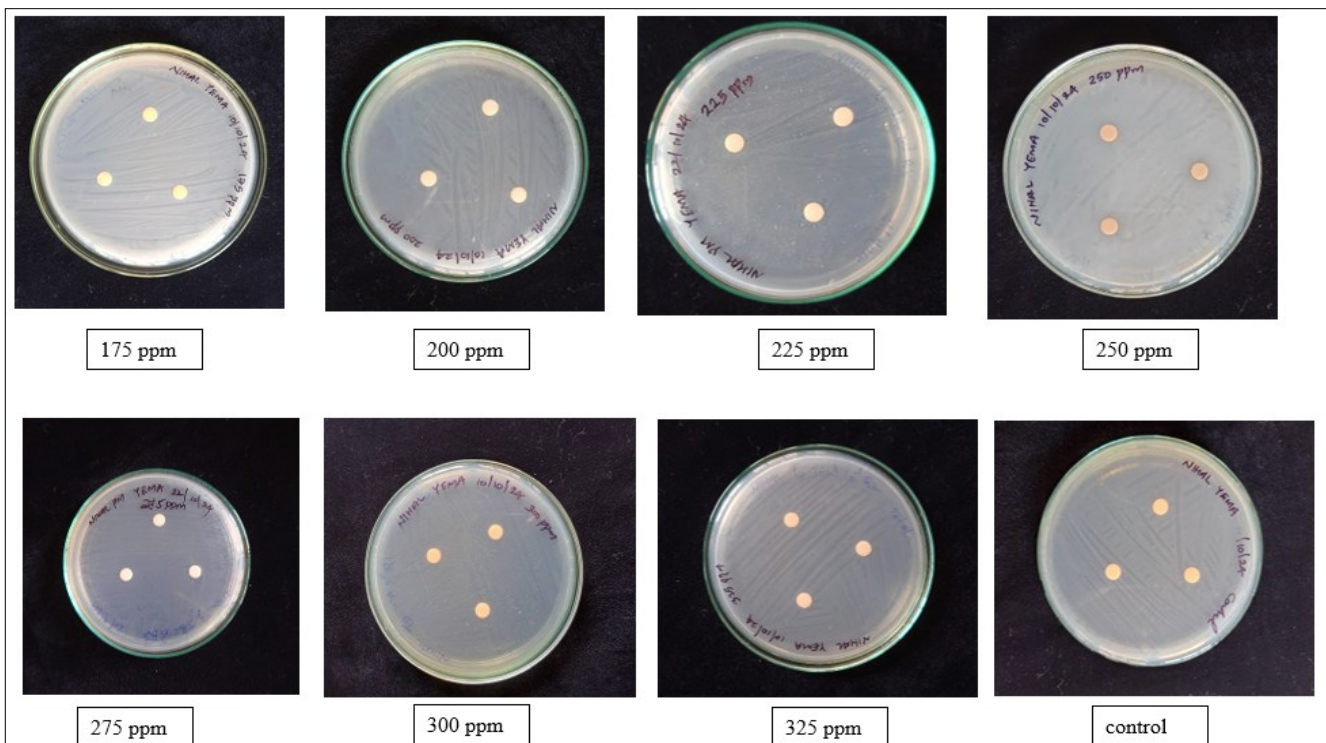
### Influence of premix herbicides on soil microbial population

Soil microbial population exhibited variations depending on the

**Table 1.** *In vitro* sensitivity of *Bradyrhizobium* to herbicide mixtures

| pendimethalin +<br>imazethapyr<br>concentration ( $\mu\text{L L}^{-1}$ ) | Growth of <i>Bradyrhizobium</i>  |        | propaquizafop +<br>imazethapyr concentration<br>( $\mu\text{L L}^{-1}$ ) | Growth of <i>Bradyrhizobium</i>  |        |
|--------------------------------------------------------------------------|----------------------------------|--------|--------------------------------------------------------------------------|----------------------------------|--------|
|                                                                          | Inhibition zone at<br>3 DAI (mm) | Growth |                                                                          | Inhibition zone at<br>3 DAI (mm) | Growth |
| 800                                                                      | Nil                              | +      | 175                                                                      | Nil                              | +      |
| 1000                                                                     | Nil                              | +      | 200                                                                      | Nil                              | +      |
| 1200                                                                     | Nil                              | +      | 225                                                                      | Nil                              | +      |
| 1400                                                                     | Nil                              | +      | 250                                                                      | Nil                              | +      |
| 1600                                                                     | Nil                              | +      | 275                                                                      | Nil                              | +      |
| 1800                                                                     | Nil                              | +      | 300                                                                      | Nil                              | +      |
| 2000                                                                     | Nil                              | +      | 375                                                                      | Nil                              | +      |
| 0 (control)                                                              | Nil                              | +      | 0 (control)                                                              | Nil                              | +      |

+: positive growth around the sterile disc; DAI- days after incubation.

**Fig. 1.** *In vitro* sensitivity of *Bradyrhizobium* to pendimethalin+ imazethapyr.**Fig. 2.** *In vitro* sensitivity of *Bradyrhizobium* to propaquizafop + imazethapyr.

type of herbicide applied and the sampling time. In general, no significant differences were observed up to 15 days after herbicide application, but distinct changes became evident by 30 DAS.

#### a) *Rhizobium*

*Rhizobium* counts remained stable before herbicide application and at 15 DAS, suggesting that the applied premix herbicides did not exert any immediate toxic effect on this symbiotic bacterium. By 30 DAS, however, distinct treatment effects were observed. Plots treated with T<sub>2</sub> (7.48 log<sub>10</sub> CFU g<sup>-1</sup>) and T<sub>1</sub> (7.45 log<sub>10</sub> CFU g<sup>-1</sup>) recorded significantly higher *Rhizobium* population, whereas T<sub>4</sub> and T<sub>5</sub> (6.24 log<sub>10</sub> CFU g<sup>-1</sup>) and T<sub>8</sub> (6.48 log<sub>10</sub> CFU g<sup>-1</sup>) showed comparatively lower counts (Table 2).

The enhanced population under pendimethalin + imazethapyr (T<sub>1</sub> and T<sub>2</sub>) could be attributed to a favourable rhizospheric environment resulting from early and effective weed suppression, which likely reduced competition for nutrients, improved light interception and minimised root-zone disturbance (14). In addition, certain soil microbes, including some *Bradyrhizobium* strains, may possess metabolic pathways enabling partial degradation of pendimethalin, thereby using it as a carbon source and gradually increasing their number (15). Certain microbial communities can tolerate or even utilise the molecules of herbicides or their byproducts as additional carbon or energy sources, causing a temporary increase in numbers (16, 17). Conversely, the lower rhizobial counts in T<sub>4</sub>, T<sub>5</sub> and T<sub>8</sub> may have resulted from subtle herbicide-induced stress or indirect effects such as shifts in microbial community composition, increased competition from non-symbiotic microbes, or changes in root exudation patterns that are less favourable for rhizobial proliferation. Similar differential responses have been reported in mungbean rhizospheres, where pendimethalin + imazethapyr had minimal effect on fungal and actinomycete population, indicating that the impact of these herbicides is both species- and context-dependent (18).

Maintaining robust *Rhizobium* population is crucial for ensuring effective nodulation and biological nitrogen fixation in legume-based systems. The judicious herbicide selection and application timing can result in efficient weed control without impairing symbiotic nitrogen fixation (19). However, caution is warranted, as it is demonstrated that certain herbicide stresses can impair rhizobial performance in other legume systems, such as red clover (20). The rhizobia that are associated with temperate legumes like red clover could be more susceptible to herbicides and

the acidic tropical soils may decrease the bioavailability of herbicides, suggesting that the impact of herbicides on the symbiotic nitrogen-fixing bacteria varies with the legume species, rhizobia, soil and herbicide behaviour in soil. These findings stress the need for integrated weed management strategies that sustain both crop productivity and rhizosphere microbial health. However, evaluating herbicide effects under both *in vivo* and *in vitro* conditions is essential to clearly distinguish the direct impact of herbicides on rhizobia from changes in herbicide behaviour arising from environmental factors, as well as from indirect effects mediated through the host plant.

#### b) Phosphate solubilising bacteria (PSB)

Phosphorus-solubilising bacterial population showed a remarkably consistent trend across all the treatments and sampling intervals, with the population ranging between 3.0 to 3.2 log<sub>10</sub> CFU g<sup>-1</sup> soil (Table 2). This uniformity indicated that the herbicide treatment did not impose any measurable stress on PSB population. As PSB activity is dependent on soil phosphorus availability, it can be assumed that the consistently higher P status in the experimental soil may have provided a conducive environment to the PSB population to sustain their activities irrespective of the weed management intervention.

Adequate phosphorus levels can mitigate the effect of external stress factors on PSB dynamics by reducing the dependency on microbial solubilisation processes for crop nutrient uptake (21). Moreover, the absence of significant weed-crop competition for nutrients in herbicide-treated plots may have indirectly contributed to the stability of PSB population by maintaining nutrient balance in the rhizosphere. These results suggest that when there is an adequate supply of P in the soil, herbicides applied at recommended rates may be safe for PSB population, thereby safeguarding their functional role in nutrient cycling.

#### c) Nitrogen fixing bacteria

The abundance of free-living nitrogen-fixing bacteria showed significant variation prior to herbicide application and at 30 DAS. At 30 DAS, the highest population was observed in T<sub>3</sub> (propaquizafop + imazethapyr, 4.68 log<sub>10</sub> CFU g<sup>-1</sup>), statistically comparable to T<sub>9</sub>, T<sub>7</sub>, T<sub>4</sub> and T<sub>1</sub>, while T<sub>5</sub> recorded the lowest (3.10 log<sub>10</sub> CFU g<sup>-1</sup>) (Table 2). The superior counts in T<sub>3</sub> treated plots may be attributed to effective early-season weed suppression, which reduced competition for resources and maintained a favourable rhizosphere microclimate

**Table 2.** Rhizospheric population of beneficial bacteria in the soil just before, 15 and 30 days after spraying of herbicide

| Treatments     | Microbial population (log <sub>10</sub> CFU g <sup>-1</sup> soil) |                                     |                                     |                                  |                                     |                                     |                              |                                     |                                     |
|----------------|-------------------------------------------------------------------|-------------------------------------|-------------------------------------|----------------------------------|-------------------------------------|-------------------------------------|------------------------------|-------------------------------------|-------------------------------------|
|                | Rhizobium                                                         |                                     |                                     | Phosphorus solubilising bacteria |                                     |                                     | Nitrogen fixers              |                                     |                                     |
|                | Before herbicide application                                      | 15 days after herbicide application | 30 days after herbicide application | Before herbicide application     | 15 days after herbicide application | 30 days after herbicide application | Before herbicide application | 15 days after herbicide application | 30 days after herbicide application |
| T <sub>1</sub> | 7.48 ± 0.00                                                       | 6.56 ± 0.14                         | 7.45 ± 0.12 <sup>ab</sup>           | 3.10 ± 0.17                      | 3.10 ± 0.17                         | 3.00 ± 0.00                         | 4.50 ± 0.08 <sup>b</sup>     | 4.67 ± 0.23                         | 4.24 ± 0.24 <sup>ab</sup>           |
| T <sub>2</sub> | 7.48 ± 0.00                                                       | 6.56 ± 0.14                         | 7.48 ± 0.00 <sup>a</sup>            | 3.00 ± 0.00                      | 3.10 ± 0.17                         | 3.00 ± 0.00                         | 4.72 ± 0.27 <sup>a</sup>     | 4.12 ± 0.62                         | 3.52 ± 0.66 <sup>cd</sup>           |
| T <sub>3</sub> | 7.45 ± 0.12                                                       | 7.16 ± 0.22                         | 6.90 ± 0.07 <sup>c</sup>            | 3.00 ± 0.00                      | 3.00 ± 0.00                         | 3.10 ± 0.17                         | 4.68 ± 0.23 <sup>a</sup>     | 4.24 ± 0.24                         | 4.68 ± 0.01 <sup>a</sup>            |
| T <sub>4</sub> | 7.45 ± 0.12                                                       | 6.60 ± 0.00                         | 6.24 ± 0.34 <sup>d</sup>            | 3.10 ± 0.17                      | 3.00 ± 0.00                         | 3.00 ± 0.00                         | 3.90 ± 0.37 <sup>b</sup>     | 4.24 ± 0.24                         | 4.48 ± 0.06 <sup>a</sup>            |
| T <sub>5</sub> | 7.16 ± 0.22                                                       | 6.69 ± 0.07                         | 6.24 ± 0.34 <sup>d</sup>            | 3.00 ± 0.00                      | 3.00 ± 0.00                         | 3.00 ± 0.00                         | 4.66 ± 0.30 <sup>a</sup>     | 4.52 ± 0.48                         | 3.10 ± 0.17 <sup>d</sup>            |
| T <sub>6</sub> | 7.24 ± 0.33                                                       | 6.98 ± 0.04                         | 7.08 ± 0.11 <sup>bc</sup>           | 3.10 ± 0.17                      | 3.00 ± 0.00                         | 3.00 ± 0.00                         | 4.67 ± 0.23 <sup>a</sup>     | 4.67 ± 0.23                         | 3.56 ± 0.07 <sup>cd</sup>           |
| T <sub>7</sub> | 7.01 ± 0.15                                                       | 7.07 ± 0.10                         | 6.98 ± 0.04 <sup>c</sup>            | 3.00 ± 0.00                      | 3.00 ± 0.00                         | 3.20 ± 0.17                         | 4.70 ± 0.29 <sup>a</sup>     | 4.12 ± 0.61                         | 4.49 ± 0.19 <sup>a</sup>            |
| T <sub>8</sub> | 6.95 ± 6.95                                                       | 6.60 ± 0.00                         | 6.48 ± 0.00 <sup>d</sup>            | 3.10 ± 0.17                      | 3.00 ± 0.00                         | 3.00 ± 0.00                         | 3.10 ± 0.17 <sup>c</sup>     | 4.35 ± 0.26                         | 3.90 ± 0.37 <sup>bc</sup>           |
| T <sub>9</sub> | 7.35 ± 0.49                                                       | 6.98 ± 0.47                         | 7.01 ± 0.15 <sup>c</sup>            | 3.00 ± 0.00                      | 3.00 ± 0.00                         | 3.00 ± 0.00                         | 3.10 ± 0.17 <sup>c</sup>     | 4.91 ± 0.10                         | 4.67 ± 0.23 <sup>a</sup>            |
| SEm (±)        | 0.16                                                              | 0.14                                | 0.13                                | 0.07                             | 0.05                                | 0.05                                | 0.14                         | 0.22                                | 0.17                                |
| CD (0.05)      | NS                                                                | NS                                  | 0.402                               | NS                               | NS                                  | NS                                  | 0.427                        | NS                                  | 0.496                               |

CD (0.05)-critical difference at 5 % level; NS- not significant; SEm-standard error of mean.

conducive to microbial proliferation. Previous works reported that this herbicide combination enhanced weed control efficiency (>85 %) without inducing microbial stress (22). In another study, imazethapyr was reported to temporarily alter the soil microbial communities, causing a short-term decline in microbial biomass followed by recovery within 60 days, suggesting that any herbicide-induced shifts are reversible under favourable soil conditions (23). Considering that soil contains a large bacterial population typically ranging from  $10^8$ – $10^9$  cells per gram, only about 1 % are culturable under standard laboratory conditions and observed changes in microbial counts represent only a small fraction of the total population (24). Furthermore, even herbicides with similar chemical characteristics or modes of action, can have different effects on soil microorganisms (25). Hence these results suggest that herbicide effect can be variable under field conditions when assessed using culture-based methods.

#### d) Total fungi

Fungal abundance varied significantly before herbicide application and at 30 DAS, while no significant differences were observed at 15 DAS (Table S1). At 30 DAS, the highest fungal population was recorded in plots treated with pendimethalin + imazethapyr ( $6.20 \log_{10}$  CFU  $g^{-1}$ ), followed by the weedy check ( $5.56 \log_{10}$  CFU  $g^{-1}$ ) (Fig.3). These findings suggest that the herbicide premix exerted no detrimental effects on soil fungal communities, possibly due to its mode of action being targeted primarily at plant-specific pathways, thereby leaving non-target soil microbiota unaffected.

The relatively higher counts of fungi in the weedy check could be possibly due to the increased rhizodeposition and the variety of root exudates from the uncontrolled weed flora. Weed roots release sugars, amino acids and phenolic compounds, which can act as a readily available source of carbon, supporting

saprophytic and mutualistic fungi. Such resource availability can enhance fungal proliferation, particularly in the rhizosphere. In contrast, early-season weed suppression in herbicide-treated plots could have resulted in lower organic input, leading to fungal counts that reflect only the contributions from crop roots. Compared to bacteria and actinomycetes, fungi generally show higher tolerance and survival under changing soil disturbances and demonstrate some degree of resilience (26). The overall stability of fungal population in herbicide-treated plots further supports the fact that judicious herbicide use, particularly with selective premixes, can achieve effective weed control without affecting the key soil microbial groups that are responsible for the decomposition of organic matter and nutrient cycling.

#### e) Actinomycetes

Before herbicide application, actinomycetes count ranged from 3.00 to  $3.39 \log_{10}$  CFU  $g^{-1}$ . At 15 DAS, a decline was evident in  $T_3$ ,  $T_4$ ,  $T_5$ ,  $T_6$  and  $T_7$ . By 30 DAS, higher population were maintained in  $T_1$ ,  $T_2$ ,  $T_3$  and  $T_9$  ( $3.00 \log_{10}$  CFU  $g^{-1}$ ), whereas  $T_4$ ,  $T_5$ ,  $T_6$  and  $T_7$  recorded lower counts ( $2.15 \log_{10}$  CFU  $g^{-1}$ ). Weed management practices significantly influenced actinomycetes population across all sampling intervals (Table S1). The weedy check consistently supported higher population of actinomycetes, nitrogen-fixing bacteria and fungi at 30 DAS (Fig. 4). This enrichment may be due to the lack of herbicide-induced stress, continuous addition of diverse root exudates from weeds, lack of soil disturbance and improved soil moisture retention due to the weed cover. Such conditions were known to favour the proliferation of soil microbial communities, particularly actinomycetes, which play a vital role in organic matter decomposition and secondary metabolite production. Higher microbial activity indicates herbicide breakdown, thereby reducing its persistence in soil, whereas

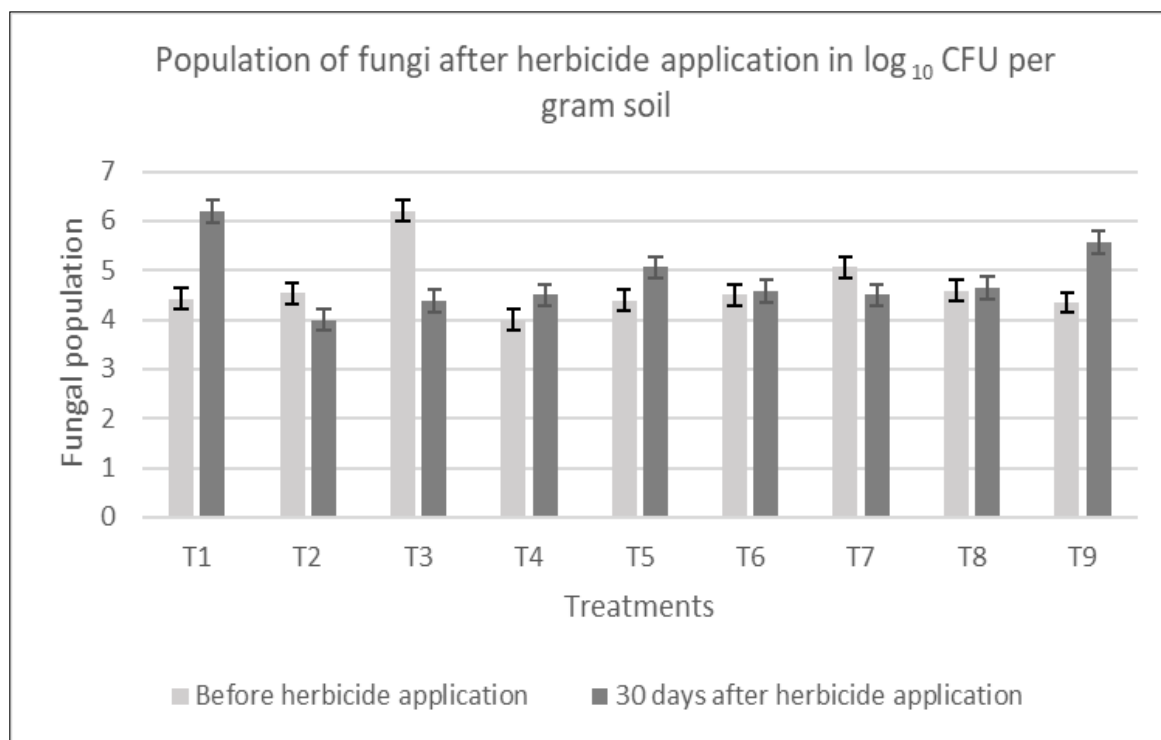


**Fig. 3.** Variation in fungal population in soil before and 30 days after herbicide application. [ $T_1$  - pre-emergence (PE) application of pendimethalin + imazethapyr at  $800 \text{ g ha}^{-1}$ ;  $T_2$  -  $T_1$  followed by hand weeding (HW) at 30 days after sowing (DAS);  $T_3$  - post-emergence (PoE) application of propaquizafop + imazethapyr at  $125 \text{ g ha}^{-1}$ ;  $T_4$  -  $T_3$  followed by HW at 40 DAS;  $T_5$  - PE pendimethalin at  $1 \text{ kg ha}^{-1}$  followed by HW at 30 DAS;  $T_6$  - PE pendimethalin at  $1 \text{ kg ha}^{-1}$  followed by imazethapyr at  $50 \text{ g ha}^{-1}$ ;  $T_7$  - PE pendimethalin at  $1 \text{ kg ha}^{-1}$  followed by propaquizafop at  $100 \text{ g ha}^{-1}$ ;  $T_8$  - HW at 15 and 30 DAS and  $T_9$  - weedy check].

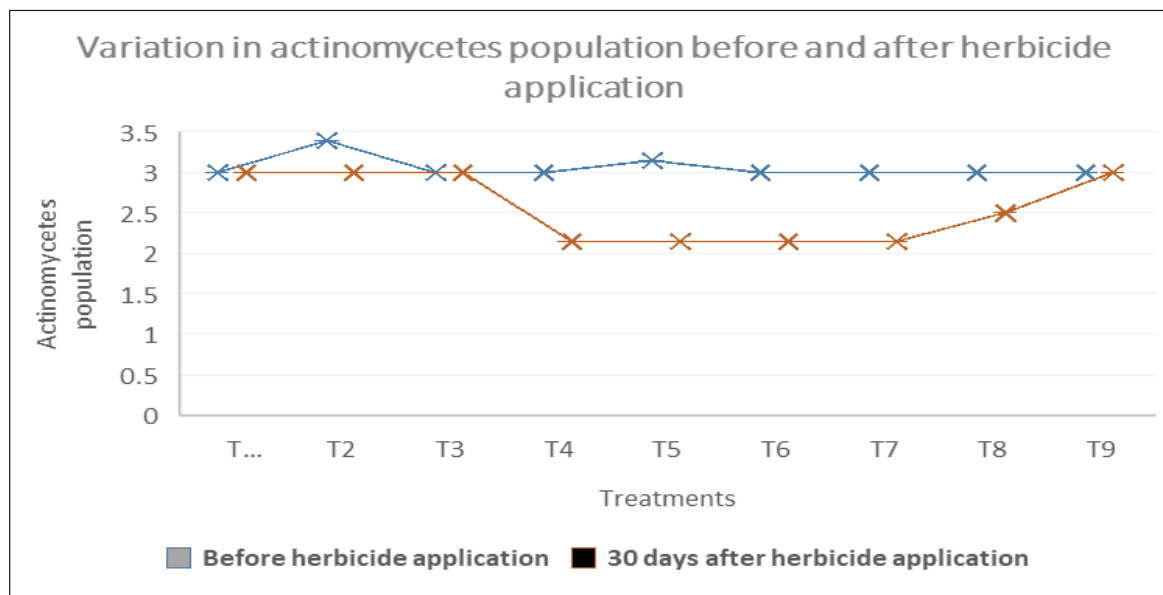


**Fig. 4.** Variation in actinomycetes population in soil before and 30 days after herbicide application. [T<sub>1</sub> - pre-emergence (PE) application of pendimethalin + imazethapyr at 800 g ha<sup>-1</sup>; T<sub>2</sub> - T<sub>1</sub> followed by hand weeding (HW) at 30 days after sowing (DAS); T<sub>3</sub> - post-emergence (PoE) application of propaquizafop + imazethapyr at 125 g ha<sup>-1</sup>; T<sub>4</sub> - T<sub>3</sub> followed by HW at 40 DAS; T<sub>5</sub> - PE pendimethalin at 1 kg ha<sup>-1</sup> followed by HW at 30 DAS; T<sub>6</sub> - PE pendimethalin at 1 kg ha<sup>-1</sup> followed by imazethapyr at 50 g ha<sup>-1</sup>; T<sub>7</sub> - PE pendimethalin at 1 kg ha<sup>-1</sup> followed by propaquizafop at 100 g ha<sup>-1</sup>; T<sub>8</sub> - HW at 15 and 30 DAS and T<sub>9</sub> - weedy check].

reduced microbial growth and activity can prolong herbicide persistence due to slower degradation processes.

## Conclusion

The study indicated that premix herbicide application, particularly pendimethalin + imazethapyr and propaquizafop + imazethapyr did not adversely affect the activity of *Bradyrhizobium*; and soil microbial population in the short term and in some cases, enhanced microbial groups such as *Rhizobium* and total fungi in black gram. Pendimethalin + imazethapyr showed no immediate adverse effects on beneficial microbes at recommended doses. In contrast, weedy check plots supported higher population of nitrogen fixing bacteria, fungi and actinomycetes at 30 DAS, likely due to the absence of herbicide stress, the release of diverse root exudates from weeds, reduced soil disturbance and improved soil moisture retention that collectively promote microbial proliferation. Although the current study offers information on the influence of herbicides on soil biological communities, their functions is complex and cannot be generalised across herbicides, soil and environmental conditions. The analysis is constrained by the short duration of the experimental period and the evaluation of only culturable microbial populations; thus, long-term studies that combine functional and molecular methods of analysis are needed to gain a deeper understanding of the long-term effects of herbicide application on soil microbial diversity.

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## Authors' contributions

PMN and EKJJ searched, collected and wrote the first draft. MA analysed the manuscript, provided the regular assistance to revise

and finalise it. SA and CBK assisted in the lab work and data tabulation. MA along with PSP and VSS reviewed and edited the manuscript. KNA critically reviewed and edited the same. All authors read and approved the final manuscript.

## Compliance with ethical standards

**Conflict of interest:** Authors do not have any conflict of interest to declare.

**Ethical issues:** None

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