Pak. J. Bot., 56(2): 765-774, 2024. DOI: http://dx.doi.org/10.30848/PJB2024-2(28)

ISOLATED BACTERIAL STRAINS EFFICIENTLY DEGRADE GLYPHOSATE UNDER DIFFERENT ENVIRONMENTAL CONDITIONS

WAQAS MOHY-UD-DIN[1,2,4*], MUHAMMAD JAVED AKHTAR[1], SAFDAR BASHIR[2*], HAFIZ NAEEM ASGHAR[1], MUHAMMAD FARRAKH NAWAZ[3] AND FENG CHEN[4]

[1]*Institute of Soil and Environmental Sciences, University of Agriculture, Faisalabad 38040, Pakistan*
[2]*Department of soil and Environmental Sciences, Ghazi University, D. G. Khan, 32200, Pakistan*
[3]*Department of Forestry & Range Management, University of Agriculture, Faisalabad 38040, Pakistan*
[4]*Institute of Marine and Environmental Technology, University of Maryland Center for Environmental Science, Baltimore, MD 21202, United states of America*
[*]*Corresponding author's email:mohyuddin.waqas5@gmail.com; sabashir@gudgk.edu.pk*

Abstract

Glyphosate (GLX) is a crucial organophosphonate herbicide applied to eliminate unwanted, grasses and herbaceous plants in various vegetation management situations. Its widespread and consequently application is causing environmental pollution, there is a need to remove it from the environment using an eco-friendly and cost-effective method. Biodegradation is an effective method for the removal of GLX. But various environmental factor effecting is degradation after its applications such as aerobic and anaerobic environment, different pH and temperature, soil water contents and soil textures. Glyphosate resistant bacterial strains have been isolated from GLX-contaminated soil which were proficient to utilize GLX as a carbon source. High Pressure Liquid Chromatography instrument was used to determine the rate of GLX biodegradation in liquid media and soil under different environmental conditions. The five out of eleven strains such as WAG2 (*Serratia liquefaciens*), WAG4 (*Klebsiella variicola*), WAG45 (*Enterobacter cloacae*), WAG9 (*Pseudomonas aeruginosa*), and WAG11 (*Enterobacter ludwigii*) were proficient in average degraded 95 to 98 mg kg^{-1} glyphosate in soil followed by liquid media 93 to 96 mg kg^{-1} within 28 days of incubation in different environmental factors. The optimal circumstances for the degradation of GLX were found to be aerobic, pH 7, 40˚C, soil water content at 10% and sandy loam texture showed maximum degradation at 100 mg kg^{-1} compared to 200 mg kg^{-1} concentration of GLX. This study demonstrates the potential of isolated bacterial strains for efficient degradation of GLX, which can be exploited for remediation of GLX in contaminated soil.

Key words: Biodegradation, Bioremediation, Herbicides, Organophosphate, Rhizobacteria.

Introduction

Glyphosate (GLX) is one of the broadly applied organophosphorus herbicides against annual and perennial weeds, silviculture and urban zones. Glyphosate is an important constituent of post-emergent and non-selective weed killers used to protect crops against invasive grasses, woody plants, annual broad-leaved weeds. Glyphosate's possible mode of action becomes it an interesting herbicide (Singh *et al*., 2020b). The worldwide GLX market was $23.97 billion in 2016, and with a forecasted production rate 6.05%, it is expected to achieve $34.10 billion in 2022. In Pakistan, nearly 1,100 tons of glyphosate was imported in 2015, while this figure increased to 1,700 tons in 2016 (The Express Tribune, 2017). Glyphosate is the sole weed killer that inhibits aromatic amino acid production in the shikimate pathway by targeting 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS). Glyphosate inhibits the production of important protein and secondary metabolites, as well as the key energy routes in rhizosphere microbes and plants. According to previous investigation, GLX changes texture of soil and microbial diversity by decreasing community of microorganisms and boosting the phytopathogenic fungus population (French *et al*., 2021). This herbicide is thought to be safer compared to others, although excessive usage has long-term impacts on the natural environment and individuals. Furthermore, its extensive herbicidal activity and the introduction of GLX-resistant crops (e.g., canola, sugarcane, maize, cotton, and soybean) are the primary causes of its overuse. Due to a lack of understanding about the herbicide's usage, it has accumulated in both terrestrial and aquatic habitats. Because GLX may be adsorbed on clay particles, it often accumulates in the vadose zone. As a result, it is often found in surface and ground water, by run-off and leaching process. Glyphosate not only disturbed the microbial communities but also effects on vertebrates such as silver catfish (Sharma et al., 2019). When glyphosate is used again and again, beneficial microorganisms in the soil, such as rhizobacteria, saprophytic, mycorrhizal fungi, and nitrogen-fixing bacteria, are wiped out from the soil (Zulfiqar *et al*., 2023). Glyphosate was designated as "Category 2a" by the International Agency for Research on Cancer (IARC), which means it is likely carcinogenic to humans. Glyphosate has been shown in the literature to be a carcinogenic substance that may induce organ failure in non-mammalian species by inhibiting acetylcholinesterase and producing oxidative stress (Mesnage *et al*., 2015). Because the effect of GLX and GLX-comprising herbicides on soil microbial population, biodegradation and bioremediation techniques are needed for the cleaning of GLX polluted soil. Pesticides may be removed using a variety of treatment methods. For example, biodegradation, photodegradation, oxidation and adsorption approaches. Glyphosate is predominantly degraded in soil by bacteria and fungi, which use it as a carbon source, resulting in the synthesis of amino-methylphosphonic acid, or as a phosphorus source produce glycine (Singh *et al*., 2020a). Bacteria are among the most adaptable and diverse creatures in terms of nutritional needs (Mohy-Ud-Din *et al*., 2023). The development of a low-cost, ecologically friendly bioremediation technology based on GLX-

degrading bacteria is a potential strategy for remediating GLX-contaminated soils. Plant growth promotion rhizobacteria are present almost all over in the soil that, given ideal circumstances under biotic and abiotic stresses, may directly or indirectly promote plant growth and protect the plants from pathogenic diseases (Oleńska *et al*., 2020). Environmental factors such as abiotic and biotic environments, pH, temperature, soil texture, and soil water content all had a significant impact on GLX biodegradation. The objective of this study was to evaluate the rate of GLX biodegradation in different environments i.e., anaerobic, and aerobic conditions, different pH and temperature, soil texture and soil water content. For this objective, Isolation of rhizobacterial strains with the ability to break down GLX in liquid medium and soil were used.

Material and Methods

Eleven sites (28°26'15.7"N 70°35'14.5"E) were used to isolates the bacteria from rhizosphere soil (10-year history of GLX application) through an enrichment technique that used GLX as a sole source of carbon. The suspension was developed by getting 20 g rhizosphere soil and mineral salt medium (MSM) 100 ml properly mixed, set in an orbital shaking incubator under 28±2˚C for 24 hours at 150 rev min^{-1}. The MSM consisted of K_2HPO_4 0.1 g, $CaSO_4$ 0.05 g, $(NH_4)2SO_4$ 0.1 g, $FeSO_4 \cdot 7H_2O$ 0.01 g, $MgSO_4 \cdot 7H_2O$ 0.2 g and distilled water 1.0 L, pH 7.0. After that, solid soil particles were given a one-hour settling time, supernatant collected, and filtered through, Whatman filter paper (No. 42). The MSM in 50 mL conical flasks were sterilized for 20 minutes at 121˚C. Glyphosate 200 mg L^{-1} concentration was filter sterilized and added in MSM media. Then, 1 mL aliquots from the above prepared soil mixture were added in the 9 ml MSM media spiked with GLX, incubated at 28±2˚C at 150 rev min^{-1} for fourteen days. This practice was repetitive twice, then 0.1 mL of inoculum added in 10 mL (MSM) spiked with 200 mg L^{-1} GLX in separate sterilized flask, incubated at 28±2˚C at 150 rev min^{-1} for 7 days. Finally, 200-300 µl culture samples were taken and spread on agar media spiked with filter sterilized GLX to get purified GLX tolerant bacterial isolates.

Isolated bacterial strains were tested through incubation study in Soil Microbiology and Biochemistry lab, Institute of Soil and Environmental Sciences, University of Agriculture Faisalabad, to evaluate the GLX biodegradation at different concentrations (100 mg L^{-1} and 200 mg L^{-1}) under aerobic and anaerobic, different pH and temperatures in liquid media and soil conditions. Glyphosate biodegradation was also evaluated in different soil water contents and soil texture. For the evaluation of GLX biodegradation two experimental sets 100 mg L^{-1} and 200 mg L^{-1} were prepared which include the following layout (1) non-inoculated control spiked with GLX (2) inoculation of isolated bacterial strains separately in each flask (for liquid media) and jar (having capacity for 300 g soil) spiked with GLX to reach the concentration up to 100 and 200 mg kg^{-1}.

Evaluation of biodegradation of glyphosate in liquid media and soil: Glyphosate optimization and biodegradation was assessed in broth culture and soil under aerobic and anaerobic conditions. The soil samples (pesticide free) were same in all the experiments except for different soil texture experiment. The physiochemical properties of soil are shown in (Table S1). The MSM media, soil and flasks were autoclaved for 20 minutes at 121˚C. While jars were sterilized with ethanol (70%) and washed with autoclaved deionized water. The flasks containing 50 ml MSM media and jars filled with 300 g soil were spiked with GLX (100 mg L^{-1} and 200 mg L^{-1}) and 800 µl inoculum of isolated bacterial strains (OD600=0.80) were inoculated separately in each treatment. The abiotic degradation of GLX was checked using the same quantity of MSM media and soil (without inoculation) spiked with two concentrations of GLX. One batch of flasks was incubated on shaker at 150 rev min^{-1} under shaking condition (aerobic), while another set of flasks placed in an incubator screwed with cork under static condition (anaerobic) at 28±2˚C. In case of jar, soil was mixed with sterilized spatula after 24 hours to maintain aerobic environment, while in anaerobic environment jar were sealed with polythene sheet and placed in incubator at 28±2˚C. Irrigation was applied in jar soil to maintain moisture level. To maintain accuracies experiment sets were incubated with three replicates for 28 days.

Table S1. Physicochemical properties of the soil.

Parameters	Soil
Sand (g kg^{-1})	520
Slit (g kg^{-1})	329
Clay (g kg^{-1})	150
CEC (cmol kg^{-1})	6.2
pH	7.8
Organic C (g kg^{-1})	6
Organic matter (g kg^{-1})	8.1
C:N ratio	10.1
Total mineral N (mg kg^{-1})	8.2
K (mg kg^{-1})	88.1
P (mg kg^{-1})	3.5

Evaluation of glyphosate biodegradation under different pH level in liquid media and soil: The optimization and biodegradation of two different concentration of GLX was investigated at different levels of pH in liquid media and soil. For this objective, four batch of liquid media (pH 5, 6, 7 and 8) and four batch of soil (pH 5, 6, 7, and 8) were maintained. The pH was adjusted by adding diluted HCl or NaOH solution. The flasks containing 50 ml MSM media (pH 5, 6, 7 and 8) and jars filled with 300 g soil (pH 5, 6, 7 and 8) were spiked with GLX (100 mg L^{-1} and 200 mg L^{-1}) and 800 µl inoculum of isolated bacterial strains (OD600=0.80) were inoculated separately in each treatment and placed into incubator at 28±2˚C. The abiotic degradation of GLX was checked using the same quantity of MSM media and soil (without inoculation) spiked with two concentrations of GLX and placed into incubator at 28±2˚C for 28 days of incubation. Irrigation was applied in jar soil to maintain moisture level. The experiment was run in triplicate.

Evaluation of glyphosate biodegradation under different temperature in liquid media and soil: The optimization and biodegradation of GLX was investigated at different temperature in liquid media and soil. For this objective, the flasks containing 50 ml MSM media and jars filled with 300 g soil were spiked with GLX (100 mg L^{-1} and 200 mg L^{-1}) and 800 µl inoculum of isolated bacterial strains (OD600=0.80) were inoculated separately in each treatment and placed into incubator at 20, 30, 40 and 45 ± 2˚C. The abiotic degradation of GLX was checked using the same quantity of MSM media and soil (without inoculation) spiked with two concentrations of GLX and placed into incubator at 20, 30, 40 and 45 ± 2˚C for 28 days of incubation. Irrigation was applied in jar soil to maintain moisture level. The experiment was run in triplicate.

Evaluation of glyphosate biodegradation in different soil water contents: Bacterial strains were evaluated by GLX degradation in soil under different soil water contents. For this objective, the jar were filled with 300 g soil were spiked with GLX (100 mg L^{-1} and 200 mg L^{-1}) and 800 µl inoculum of isolated bacterial strains (OD600=0.80) were inoculated separately in each treatment, deionized water was used to obtain water contents (w/v) up to 5, 10, 15 and 20% and placed into incubator at 28 ± 2˚C with triplicate along with control flasks (without inoculation) for 28 days. The experiment was run in triplicate.

Evaluation of glyphosate biodegradation in different soil texture: Glyphosate biodegradation was evaluated under four different texture of soil such as clay loam, loam, sandy loam and sandy clay loam. Jars were filled with 300 g soil, spiked with 100 mg L^{-1} and 200 mg L^{-1} GLX (filter sterilized 0.22 µm), 800 µl inoculum of isolated bacterial strains (OD600=0.80) were applied separately in each treatment, deionized water was used to maintain moisture level and placed into incubator at 28 ± 2˚C with triplicate along with control flasks (without inoculation) for 28 days.

Analytical procedure for glyphosate determination: The 10 ml of liquid media and 10 g of soil took form flask and jar (spiked with GLX) after 14 and 28 days in case of anaerobic and aerobic conditions while remaining experiment samples were taken after 28 days. The obtain material (liquid media and soil) was added into 50 ml centrifuge tubes then added with 20 ml 0.01 M KH_2PO_4 and shake for 2 hours on rotary shaker. This process followed by centrifugation at 8000 g for 10 minutes after which, the supernatants was filtered through syringe filter 0.22 µm and transferred to derivatization process (Garba *et al*., 2018). For derivatization process followed by addition of 1 ml in centrifuge tubes (25 ml) then followed by addition of 1 ml 0.02 M FMOC-Cl and 2 ml 0.05 M borate buffer. The mixture was shaken at 4 g for 1 hour on end-to-end shaker after which 2 ml diethyl ether was added to each tube and vortex for 2 minutes to remove unreacted FMOC-Cl. Organic layer was discarded and the aqueous solution transferred to GC vials for further analysis (Garba *et al*., 2018). All the chemicals were analytical grade. Acetonitrile (HPLC grade), diethyl ether, GLX (99.7%) and FMOC-Cl (97%) were obtained from Sigma Aldrich® (Germany).

Glyphosate residue was determined by Sykam High Pressure Liquid Chromatography (HPLC) system (Germany) equipped with UV/Vis Detector (Model S 3345) DAD (Diode Array Detector); pump system (Model S1125G), analytical column was reverse-phase C18 (Sykam) and column oven (Model S 4120). Acetonitrile (HPLC grade) and 0.05 M KH_2PO_4 mixture (30:70 v/v) was used in isocratic mode for mobile phase. The running time was 15 minutes with flow rate of 0.7 ml/min and column temperature was 40˚C, while the injection volume was 20 µl. For the determination of GLX residue through HPLC-DAD detector, two wavelengths 210 and 315 nm were used. The retention time of GLX was 3 min. Data were obtained and investigated using clarity chromatography computer software. For quantification, calibration curve constructed using known quantities of GLX standard (0.001 to 300 mg kg^{-1} concentration) (Garba *et al*., 2018). Glyphosate biodegradation was checked after 14 and 28 days of application. Glyphosate degraded concentration was found by the following formula.

$$\text{Glyphosate degraded mg kg}^{-1} = T_0 - T_1$$

where T_0 represented glyphosate concentration at 0 hour, T_1 represented glyphosate concentration present in sample.

Statistical analysis

All the experimental set-up was carried out in triplicates under completely randomized design in factorial design. Treatment means were compared using Tukey's HSD test at $p<0.05$ using XLSTAT software (Hasanuzzaman *et al*., 2019; Hossain *et al*., 2020). Principal component analysis (PCA) was used to check the positive and negative correlation between isolated bacterial strains and parameters using Origin Pro 2022b Software (Chi *et al*., 2022; Dong *et al*., 2022). Pearson's correlation was applied to analyze the correlation among different parameters on RStudio 2022.07.1 Build 554.

Bacterial identification: Freshly prepared colonies (48 h) were sent to Macrogen, Korea for the identification of the isolated bacterial strains. Genomic DNA was isolated according to the Macrogen protocol and used as a template for polymerase chain reaction (PCR).The forward primer consisting of the following base pairs 27F 5' (AGA GTT TGA TCM TGG CTC AG) 3' and reverse primer consisting of the following base pairs sequence 1492R 5' (TAC GGY TAC CTT GTT ACG ACT T) 3' (Pandey *et al*., 2021). The PCR conditions were 94˚C for 5 minutes, 94˚C for 1 minute, 50˚C for 1 minute, followed by 30 cycles of 72˚C for 90 seconds, with a final step at 72˚C for 10 minutes. The result of sequencing was submitted to GenBank for BLAST analysis to generate the accession number (Benson *et al*., 1998).

Results and Discussion

The results of our study in liquid medium indicated that highest degradation of GLX was observed in aerobic environment by WAG2 84.05 and 31.88 mg kg^{-1} followed

by WAG5 83.83 and 31.20 mg kg^{-1} strains respectively, at level 1 and 2 after 28 days of incubation (Fig. 1). Minimum biodegradation was observed by the treatment inoculated with WAG3 59.26 and 31.8 mg kg^{-1} respectively, at both levels after 28 days. While at anaerobic environment (liquid medium), biodegradation of GLX was not enough as compared to aerobic conditions. Highest GLX degradation was observed by WAG2 57.74 and 20.37 mg kg^{-1} at both levels after 28 days of incubation. WAG3 observed least glyphosate degradation that was 28.98 and 0.49 mg kg^{-1} respectively, at both levels of GLX. In control (abiotic), GLX degradation was 0.59 and 0.20 mg kg^{-1} respectively at 100 and 200 mg kg^{-1} after 28 days of incubation under aerobic condition. Significant difference was observed between 100 and 200 mg kg^{-1} of glyphosate at $p<0.05$.

Results of our study in soil revealed that at 100 mg kg^{-1} of GLX, bacterial strains WAG11 followed by WAG9 degraded 99.6 and 99.36 mg kg^{-1} respectively, after 28 days of incubation under aerobic condition. Bacterial strains WAG2, WAG4 and WAG5 average degraded 95.31 mg kg^{-1} of GLX after 28 days. At 200 mg kg^{-1} of GLX average degraded concentration was 34.08 mg kg^{-1} observed by WAG2, WAG4, WAG5, WAG9 and WAG11 after 28 days. In control (abiotic soil), maximum degraded concentration of GLX was 0.59 and 0.20 mg kg^{-1} after 28 days of incubation at both levels. Significant difference was observed between 100 and 200 mg kg^{-1} of GLX at $p<0.05$ (Table 1).

Results concluded that highest biodegradation of GLX was observed in aerobic soil followed by inoculum as compared to anaerobic conditions (soil and liquid media) at 100 mg kg^{-1}. The current study lined with (Sims and Kanissery, 2019; Wang *et al.*, 2018). The chlorpyrifos bioavailability for biodegradation was very less due to its low solubilization in liquid (2 mg L^{-1}) and having higher affinity with soil particle and organic matter (Chishti *et al.*, 2013). Kumar and Philip (2006) investigated the breakdown of endosulfan by three bacterial species, *Staphylococcus* sp., *Bacillus circulans*-I and *Bacillus circulans*-II, in both mixed and pure cultures. After four weeks of incubation in mixed culture, maximum degradation 71.82% and 76.04% of endosulfan was detected in aerobic and facultative anaerobic conditions respectively. Pesticide concentration, solubility, and availability to microorganisms are critical variables influencing the pace and degree of bioremediation. Farhan *et al.* (2021) stated that *P. aeruginosa* was degraded chlorpyrifos up to 75 mg L^{-1} concentration in MSM, but greater doses were deleterious to the bacterium development and growth. Though, Hu *et al.* (2020) revealed that the chlorpyrifos concentration had no influence on degradation rate, but when the chlorpyrifos contamination reached to 200 mg L^{-1}, bacteria stopped the chlorpyrifos degradation and proliferate leisurely. However, our study showed that degradation at 200 mg kg^{-1} of GLX is reduced due to the possible toxic effect of GLX on bacterial growth compared to 100 mg kg^{-1}. Results concluded that isolated GLX tolerant bacterial strains can degrade 78-98% at 100 mg kg^{-1} of GLX in liquid media and soil within 28 days. Significant difference was observed between 100 and 200 mg kg^{-1} of GLX at $p<0.05$.

During the degradation process pH is one of the important factors responsible for the degradation of GLX. Results of our pH dependent experiment as shown in (Fig. 2) revealed that least biodegradation was observed in control (uninoculated) at pH 7 in liquid culture media that was 0.30 and 0.21 mg kg^{-1} and soil presented in (Table S2), 0.38 and 0.24 mg kg^{-1} at 100 and 200 mg kg^{-1} respectively, after 28 days. While at 100 mg kg^{-1} of GLX all the inoculated treatments showed biodegradation but at the pH 7 revealed highest biodegradation rate. Bacterial strains WAG2, WAG4, WAG5, WAG9 and WAG11 average degraded 85.48 mg kg^{-1} of GLX in liquid culture media after 28 days. At 200 mg kg^{-1} of GLX average degradation was observed by 41.43 mg kg^{-1} in the respective isolated strains as shown in (Fig. 2b). However, in soil all the inoculated treatments degraded 79.20-98.12 mg kg^{-1} of GLX at pH 7 after 28 days of incubation at 100 mg kg^{-1} of GLX. At 200 mg kg^{-1} of GLX average degraded concentration was observed by 20.34 to 50.83 mg kg^{-1} through all the inoculated treatments. Significant difference was observed between 100 and 200 mg kg^{-1} of GLX at $p<0.05$.

Table 1. Glyphosate degradation with isolated bacterial strains in soil after 28 days of incubation.

	Anaerobic [a] 14 days		Aerobic [a] 14 days		Anaerobic [a] 28 days		Aerobic [a] 28 days	
Bacterial strains	100 mg kg^{-1}	200 mg kg^{-1}	100 mg kg^{-1}	200 mg kg^{-1}	100 mg kg^{-1}	200 mg kg^{-1}	100 mg kg^{-1}	200 mg kg^{-1}
Control	0.12 ± 0.01 g	0.02 ± 0.01 g	0.14 ± 0.03 j	0.05 ± 0.03 j	0.27 ± 0.04 g	0.23 ± 0.05 g	0.59 ± 0.09 h	0.2 ± 0.02 h
WAG1	5.87 ± 0.5 ef	3.17 ± 0.35 fg	28.97 ± 0.87 de	11.58 ± 0.25 i	47.85 ± 0.77 c	21.72 ± 0.42 e	74.61 ± 0.88 cd	24.04 ± 1.27 fg
WAG2	12.35 ± 0.64 bc	8.54 ± 0.37 de	42.63 ± 0.51 b	19.46 ± 0.37 h	65.43 ± 0.59 b	33.01 ± 1.31 d	94.05 ± 0.25 a	32.95 ± 1.33 e
WAG3	5.91 ± 0.59 ef	3.46 ± 0.09 fg	25.54 ± 0.55 f	11.18 ± 0.11 i	44.49 ± 1.08 c	14.08 ± 1.06 f	73.01 ± 0.94 d	19.48 ± 2.22 g
WAG4	12.61 ± 0.64 bc	8.84 ± 0.05 de	43.79 ± 1.04 b	19.76 ± 0.61 gh	66.08 ± 0.61 b	33.31 ± 1.15 d	95.98 ± 1.13 a	33.25 ± 1.31 e
WAG5	10.12 ± 0.64 cd	8.82 ± 0.82 de	43.77 ± 0.81 b	20.38 ± 0.67 gh	65.99 ± 0.5 b	33.29 ± 1.43 d	95.9 ± 0.61 a	33.23 ± 1.77 e
WAG6	5.64 ± 0.91 ef	3.67 ± 0.48 f	26.82 ± 0.1 ef	12.68 ± 0.1 i	48.25 ± 1.01 c	19.68 ± 0.07 e	71.83 ± 0.76 d	25.47 ± 2.25 f
WAG7	4.34 ± 0.89 f	3.37 ± 0.13 fg	29.59 ± 0.39 c-e	13.94 ± 0.58 i	46.61 ± 1.12 c	21.06 ± 1 e	79.73 ± 0.7 bc	25.44 ± 1.56 f
WAG8	4.47 ± 0.69 f	3.77 ± 0.37 f	32.16 ± 0.37 c	12.39 ± 0.45 i	47.25 ± 1.3 c	21.82 ± 0.55 e	81.65 ± 1.93 b	26.26 ± 2.25 f
WAG9	15.35 ± 0.64 ab	10.74 ± 0.39 cd	48.83 ± 0.92 a	21.66 ± 0.39 gh	70.82 ± 0.45 a	35.85 ± 0.51 d	99.36 ± 0.05 a	35.15 ± 1.61 e
WAG10	3.01 ± 1.44 fg	3.78 ± 0.44 f	29.81 ± 0.41 cd	11.49 ± 0.44 i	48.12 ± 0.55 c	18.1 ± 0.48 ef	75.67 ± 1.17 cd	23.06 ± 1.7 fg
WAG11	17.18 ± 0.64 a	11.42 ± 1.3 cd	50.51 ± 0.39 a	22.34 ± 0.9 g	73.25 ± 0.28 a	35.89 ± 0.64 d	99.6 ± 0.08 a	35.83 ± 0.21 e
HSD value ($p\leq0.05$)	3.4573		2.8581		4.2127		5.9172	

[a] Results represent the mean value ± standard deviation of three replications, Treatment means sharing same letter(s) within the column are statistically non-significant according to Tukey's HSD test at $p\leq0.05$

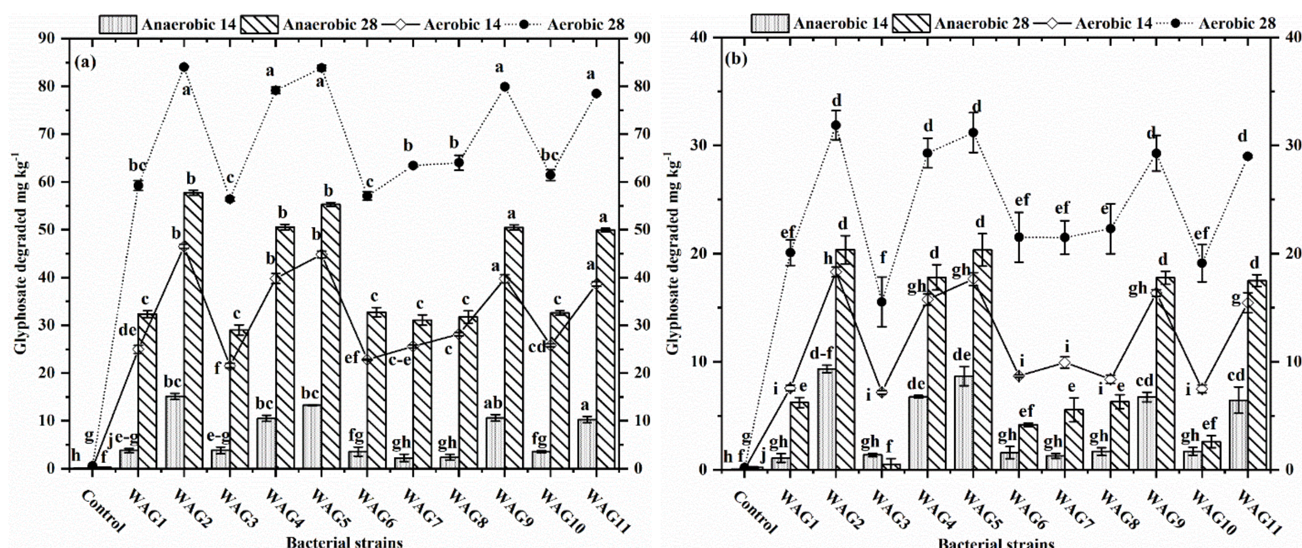

Fig. 1. Glyphosate degradation by isolated bacterial strains in liquid culture media under anaerobic and aerobic environment at two different concentrations (a) 100 mg kg^{-1} (b) 200 mg kg^{-1}. Results represent the mean value of three replicates and error bars indicate the standard deviations. Treatment means sharing the same letter (s) do not differ significantly at p<0.05.

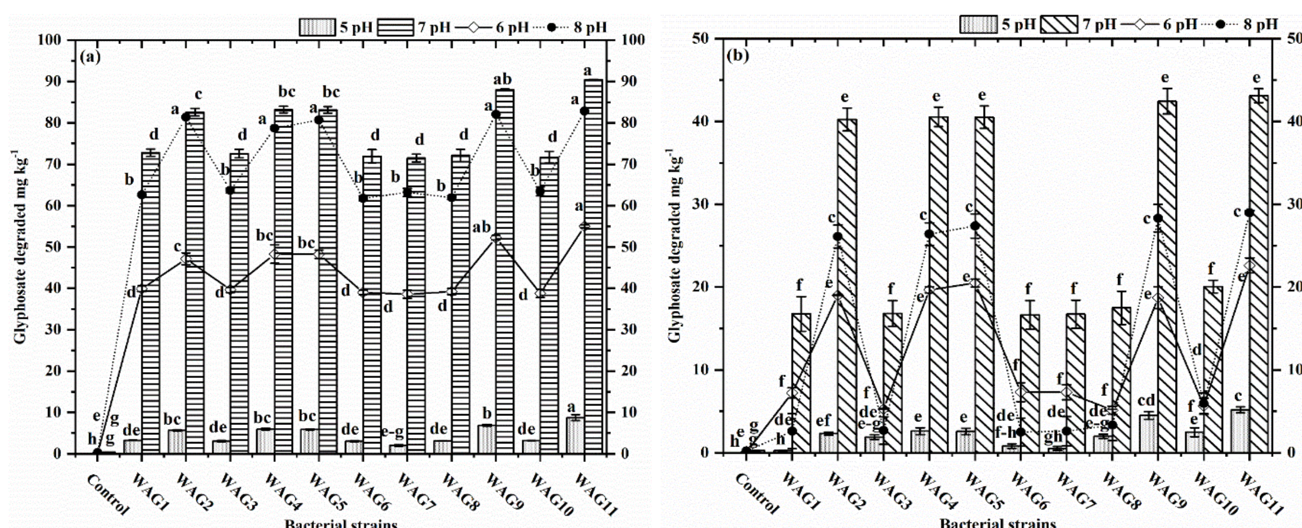

Fig. 2. Glyphosate degradation by isolated bacterial strains in liquid culture media under different pH levels at two different concentrations (a) 100 mg kg^{-1} (b) 200 mg kg^{-1}. Results represent the mean value of three replicates and error bars indicate the standard deviations. Treatment means sharing the same letter (s) do not differ significantly at p<0.05.

Table S2. Glyphosate degradation with isolated bacterial strains in soil after 28 days of incubation.

Bacterial strains	5 pH [a]		6 pH [a]		7 pH [a]		8 pH [a]	
	100 mg kg^{-1}	200 mg kg^{-1}	100 mg kg^{-1}	200 mg kg^{-1}	100 mg kg^{-1}	200 mg kg^{-1}	100 mg kg^{-1}	200 mg kg^{-1}
Control	0.03 ± 0.01 e	0.01 ± 0.01 e	0.08 ± 0.01 g	0.04 ± 0.01 g	0.38 ± 0.04 g	0.24 ± 0.04 g	0.32 ± 0.02 e	0.2 ± 0.02 e
WAG1	0.51 ± 0.07 e	0.39 ± 0.07 e	47.6 ± 0.68 d	14.18 ± 0.68 f	80.5 ± 0.89 d	24.46 ± 0.89 f	70.31 ± 0.21 b	10.32 ± 0.21 d
WAG2	6.77 ± 0.05 bc	3.27 ± 0.05 d	54.83 ± 1.42 c	26.88 ± 1.42 e	90.31 ± 0.9 c	47.95 ± 0.9 e	89.11 ± 0.29 a	33.82 ± 0.29 c
WAG3	0.29 ± 0.11 e	0.8 ± 0.11 e	47.39 ± 0.65 d	13.6 ± 0.65 f	80.29 ± 1.01 d	24.52 ± 1.01 f	71.38 ± 0.6 b	10.39 ± 0.6 d
WAG4	7.08 ± 0.1 b	3.57 ± 0.1 d	56 ± 2.24 bc	27.05 ± 2.24 e	90.95 ± 0.8 bc	48.25 ± 0.8 e	86.44 ± 0.03 a	34.12 ± 0.03 c
WAG5	7.03 ± 0.07 b	3.55 ± 0.07 d	55.97 ± 0.99 bc	27.03 ± 0.99 e	90.87 ± 0.79 bc	48.23 ± 0.79 e	88.39 ± 0.11 a	35.09 ± 0.11 c
WAG6	0.29 ± 0.06 e	0.23 ± 0.06 e	46.74 ± 0.55 d	14.13 ± 0.55 f	79.64 ± 1.61 d	24.34 ± 1.61 f	69.45 ± 0.6 b	10.21 ± 0.6 d
WAG7	1.19 ± 0.06 e	1.28 ± 0.06 e	46.29 ± 0.99 d	14.04 ± 0.99 f	79.2 ± 0.93 d	24.44 ± 0.93 f	70.93 ± 0.95 b	10.31 ± 0.95 d
WAG8	0.48 ± 0.13 e	0.88 ± 0.13 e	46.93 ± 0.74 d	13.63 ± 0.74 f	79.84 ± 1.49 d	25.2 ± 1.49 f	69.64 ± 0.4 b	11.06 ± 0.4 d
WAG9	10.54 ± 0.39 a	5.47 ± 0.39 c	59.96 ± 0.57 ab	27.67 ± 0.57 e	95.7 ± 0.19 ab	50.15 ± 0.19 e	89.79 ± 0.11 a	36.02 ± 0.11 c
WAG10	0.9 ± 0.07 e	0.22 ± 0.07 e	46.51 ± 0.99 d	13.56 ± 0.99 f	79.42 ± 1.39 d	27.78 ± 1.39 f	71.15 ± 1.08 b	13.64 ± 1.08 d
WAG11	11.78 ± 0.28 a	6.15 ± 0.28 bc	62.72 ± 0.33 a	29.63 ± 0.33 e	98.12 ± 0.08 a	50.83 ± 0.08 e	90.59 ± 0.05 a	36.7 ± 0.05 c
HSD value (p≤0.05)	1.3732		4.7882		4.8497		4.7371	

[a] Results represent the mean value ± standard deviation of three replications, Treatment means sharing same letter(s) within the column are statistically non-significant according to Tukey's HSD test at p≤0.05

Supplementary data

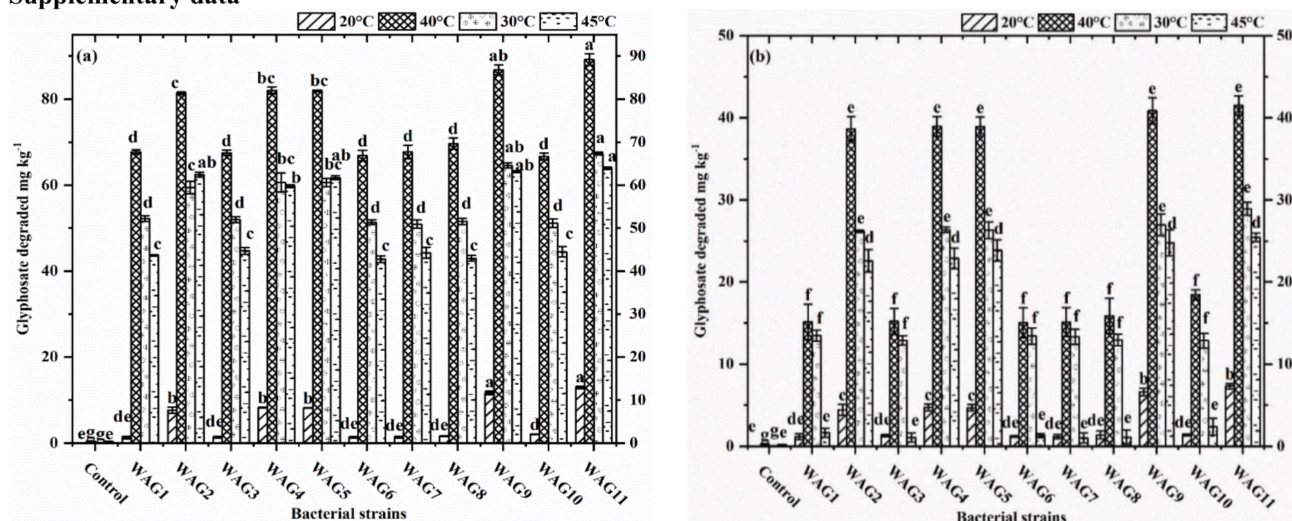

Fig. S1. Glyphosate degradation by isolated bacterial strains in liquid culture media under different temperatures at two different concentrations (a) 100 mg kg^{-1} (b) 200 mg kg^{-1}. Results represent the mean value of three replicates and error bars indicate the standard deviations. Treatment means sharing the same letter (s) do not differ significantly at $p<0.05$.

Table S3. Glyphosate degradation with isolated bacterial strains in soil after 28 days of incubation.

	20°C [a]		30°C [a]		40°C [a]		45°C [a]	
Bacterial strains	**100 mg kg^{-1}**	**200 mg kg^{-1}**	**100 mg kg^{-1}**	**200 mg kg^{-1}**	**100 mg kg^{-1}**	**200 mg kg^{-1}**	**100 mg kg^{-1}**	**200mg kg^{-1}**
Control	0.03 ± 0.01 e	0.02 ± 0.01 e	0.08 ± 0.01 f	0.04 ± 0.02 f	0.38 ± 0.04 g	0.24 ± 0.04 g	0.32 ± 0.04 e	0.2 ± 0.02 e
WAG1	1.47 ± 0.29 de	1.36 ± 0.36 de	45.67 ± 0.68 d	28.18 ± 0.63 e	79.94 ± 0.9 d	34.09 ± 2.09 f	62.6 ± 0.21 b	18.03 ± 2.1 d
WAG2	7.85 ± 0.69 b	4.62 ± 0.67 c	52.91 ± 1.42 c	40.89 ± 0.19 d	89.75 ± 0.95 c	57.59 ± 1.39 e	81.4 ± 0.31 a	41.53 ± 1.39 c
WAG3	1.54 ± 0.2 de	1.5 ± 0.12 de	45.46 ± 0.64 d	27.6 ± 0.62 e	79.73 ± 1.03 d	34.16 ± 1.55 f	63.67 ± 0.63 b	18.1 ± 1.67 d
WAG4	8.43 ± 0.1 b	4.92 ± 0.42 c	54.07 ± 1.23 bc	41.06 ± 0.29 d	90.39 ± 0.93 bc	57.89 ± 1.17 e	78.73 ± 0.03 a	41.83 ± 1.36 c
WAG5	8.37 ± 0.7 b	4.9 ± 0.37 c	54.04 ± 0.1 bc	41.03 ± 0.96 d	90.31 ± 0.8 bc	57.86 ± 1.34 e	80.68 ± 0.15 a	42.8 ± 1.46 c
WAG6	1.51 ± 0.16 de	1.39 ± 0.09 de	44.81 ± 0.57 d	28.13 ± 0.97 e	79.09 ± 1.63 d	33.98 ± 1.73 f	61.74 ± 0.62 b	17.92 ± 1.68 d
WAG7	1.55 ± 0.21 de	1.35 ± 0.26 de	44.36 ± 0.99 d	28.04 ± 0.88 e	78.64 ± 0.97 d	34.08 ± 1.71 f	63.22 ± 0.91 b	18.02 ± 1.77 d
WAG8	1.83 ± 0.13 d	1.59 ± 0.46 de	45 ± 0.74 d	27.64 ± 0.73 e	79.28 ± 1.51 d	34.83 ± 2 f	61.93 ± 0.4 b	18.77 ± 1.9 d
WAG9	11.89 ± 0.4 a	6.82 ± 0.46 b	58.03 ± 0.57 ab	41.67 ± 1.36 d	95.14 ± 0.31 ab	59.79 ± 1.55 e	82.08 ± 0.11 a	43.73 ± 1.67 c
WAG10	2.25 ± 0.7 d	1.57 ± 0.11 de	44.58 ± 1.12 d	27.57 ± 0.89 e	78.86 ± 1.41 d	37.41 ± 0.77 f	63.44 ± 1.07 b	21.35 ± 1.29 d
WAG11	13.13 ± 0.3 a	7.5 ± 0.35 b	60.79 ± 0.38 a	43.63 ± 0.76 d	97.57 ± 0.1 a	60.47 ± 0.83 e	82.88 ± 0.07 a	44.41 ± 0.24 c
HSD value ($p \leq 0.05$)	1.7322		4.8151		4.8690		4.7381	

[a] Results represent the mean value ± standard deviation of three replications, Treatment means sharing same letter(s) within the column are statistically non-significant according to Tukey's HSD test at $p \leq 0.05$

Results stated that GLX biodegradation was observed both in soil and liquid culture media among all the pH levels, but at 7 pH revealed highest degradation rate in soil followed by liquid culture media. The results of our study lined with (Sprankle *et al*., 2017). Govarthanan *et al*., (2020) stated that, liquid culture media enhanced chlorpyrifos removal and observed 93% degradation in pH 7.0 at room temperature. *B. cereus* utilized 94.47% of GLX at pH 6.0 in liquid culture media (Fan *et al*., 2012a). Dechene *et al*., (2014) evaluated GLX degradation in soils with pH 7 that were acidified to pH 5 and found that the degradation rates of imazamox, imazethapyr and imazaquin were reduced, showing that these herbicides degraded more slowly at lower pH values. Furthermore, an elevation in pH creates a more suitable environment for the growth of microbiota, mostly bacteria, which are responsible for degrading xenobiotics in the soil. Soil liming improve soil pH, enhance microbiological activity and the rates of degradation of different pesticides such as GLX (Arantes *et al*., 2011), chlorsulfuron and fenamiphos (Neina, 2019). According to Singh *et al*., (2003) soils with neutral or alkaline pH have increased microbial population stability, allowing the microorganism to swiftly adapt to breakdown the same chemical with successive applications. Higher pH levels improve the availability of certain nutrients, which encourages the formation and releasing of catalytic enzymes in the soil, which may aid in the breakdown of imidazolinones (Gehrke *et al*., 2021).

Temperature is an important factor in bioremediation of xenobiotics. The current study was focused on biodegradation of GLX under different temperatures in liquid culture media and soil. Temperature conditions were optimized in a range of 20-45˚C. In liquid culture media results revealed least biodegradation of GLX was observed at 20˚C. At 40˚C followed by 45˚C degradation of GLX was maximum in all the inoculated treatment except control (abiotic). Isolated bacterial strains WAG2, WAG4, WAG5, WAG9 and WAG11 degraded 81.40 to 89.21 and 36.64 to 41.51 mg kg^{-1} respectively, at 100 and 200 mg kg^{-1} of GLX as shown in (Fig. S1). While WAG1, WAG3, WAG6, WAG7, WAG8, and WAG10 average degraded 66.65 to 69.64 and 15.03 to 18.46 mg kg^{-1} respectively, at 100 and 200 mg kg^{-1} of GLX. The best glyphosate breakdown occurred among 30 to 45˚C, which showed a degradation of at least 70%.

Table S4. Glyphosate degradation with isolated bacterial strains in different water contents in soil after 28 days of incubation.

	Soil content [a] 5% w/v		Soil content [a] 10% w/v		Soil content [a] 15% w/v		Soil content [a] 20% w/v	
Bacterial strains	**100 mg kg^{-1}**	**200 mg kg^{-1}**	**100 mg kg^{-1}**	**200 mg kg^{-1}**	**100 mg kg^{-1}**	**200 mg kg^{-1}**	**100 mg kg^{-1}**	**200 mg kg^{-1}**
Control	18.68 ± 0.22 de	7.91 ± 0.05 f	27.72 ± 0.53 f	12.06 ± 0.23 g	24.36 ± 0.6 f	6.69 ± 1.33 g	5.86 ± 0.01 fg	0.11 ± 0.08 g
WAG1	45.93 ± 2.12 b	10.92 ± 0.81 f	68.46 ± 0.28 c	16.11 ± 1.02 g	60.2 ± 0.45 c	15.64 ± 2.24 d	26.4 ± 0.68 cc	6.51 ± 0.76 fg
WAG2	72.36 ± 2.64 a	29.96 ± 0.96 c	88.98 ± 0.16 ab	53.13 ± 4.38 d	78.36 ± 0.64 ab	39.13 ± 1.55 fg	55.22 ± 0.68 a	38.49 ± 0.12 b
WAG3	46.28 ± 2.79 b	11.06 ± 0.59 ef	68.81 ± 0.55 c	21.38 ± 5.06 fg	61.27 ± 0.65 c	15.7 ± 1.85 de	21.69 ± 0.65 cd	5.94 ± 0.42 fg
WAG4	71.59 ± 2.11 a	25.73 ± 2.4 cd	96.05 ± 0.37 a	48.25 ± 0.08 de	76.34 ± 0.24 a	39.43 ± 1.57 de	56.64 ± 1.62 a	38.66 ± 0.4 b
WAG5	74.05 ± 2.42 a	26.34 ± 2.23 cd	91.05 ± 0.86 ab	48.87 ± 0.18 de	79.57 ± 1.15 ab	40.4 ± 1.69 g	55.33 ± 0.7 a	36.64 ± 1.4 b
WAG6	45.63 ± 2.33 b	7.53 ± 1.97 f	68.16 ± 0.09 c	15.28 ± 1.19 g	59.34 ± 0.65 c	15.52 ± 1.82 fg	25.54 ± 1.28 c	8 ± 1.39 ef
WAG7	45.37 ± 2.57 b	11.55 ± 0.2 ef	67.9 ± 0.29 c	21.23 ± 4.9 fg	60.82 ± 0.75 c	15.62 ± 1.94 g	23.81 ± 1.32 c	6.37 ± 0.98 fg
WAG8	46.67 ± 2.83 b	11.66 ± 0.44 ef	69.2 ± 0.55 c	16.2 ± 1.38 g	59.53 ± 0.34 c	16.37 ± 2.01 de	25.09 ± 0.98 c	10.46 ± 1.91 ef
WAG9	70.76 ± 1.87 a	28.27 ± 2.11 c	96.5 ± 0.66 a	50.8 ± 0.31 de	79.68 ± 0.37 a	39.4 ± 0.11 fg	59.19 ± 0.97 a	39.28 ± 1.36 b
WAG10	46.7 ± 3.4 b	11.03 ± 0.67 ef	69.23 ± 1.08 c	18.14 ± 0.77 fg	61.04 ± 1.11 c	13.68 ± 0.35 e	24.67 ± 0.91 c	14.76 ± 0.52 de
WAG11	68.22 ± 0.56 a	25.35 ± 0.65 cd	84.84 ± 0.79 b	42.48 ± 0.24 e	71.05 ± 1.01 b	36.23 ± 2.14	55.65 ± 1.01 a	37.38 ± 2.33 b
HSD value ($p \leq 0.05$)	7.6835		10.108		10.108		7.1104	

[a] Results represent the mean value ± standard deviation of three replications, Treatment means sharing same letter(s) within the column are statistically non-significant according to Tukey's HSD test at $p \leq 0.05$

Table S5. Glyphosate degradation with isolated bacterial strains in different texture of soil after 28 days of incubation.

	Clay loam [a]		Loam [a]		Sandy loam [a]		Sandy clay loam [a]	
Bacterial strains	**100 mg kg^{-1}**	**200 mg kg^{-1}**	**100 mg kg^{-1}**	**200 mg kg^{-1}**	**100 mg kg^{-1}**	**200 mg kg^{-1}**	**100 mg kg^{-1}**	**200 mg kg^{-1}**
Control	0.7 ± 0.12 k	0.4 ± 0.12 k	0.27 ± 0.04 g	0.23 ± 0.05 g	0.59 ± 0.09 h	0.2 ± 0.02 h	0.14 ± 0.03 j	0.05 ± 0.03 j
WAG1	12.29 ± 0.26 g-i	8.96 ± 0.35 j	53.63 ± 0.77 c	27.5 ± 0.42 e	74.74 ± 0.87 cd	29.82 ± 1.27 fg	34.75 ± 0.87 de	17.36 ± 0.25 i
WAG2	20.06 ± 0.64 bc	14.32 ± 0.37 e-h	71.22 ± 0.59 b	38.79 ± 1.31 d	94.19 ± 0.26 a	38.74 ± 1.33 e	48.41 ± 0.51 b	25.25 ± 0.37 h
WAG3	12.34 ± 0.46 f-i	9.24 ± 0.09 j	50.28 ± 1.08 c	19.86 ± 1.06 f	73.14 ± 0.95 d	25.26 ± 2.22 g	31.32 ± 0.55 f	16.97 ± 0.11 i
WAG4	20.32 ± 0.64 b	14.62 ± 0.05 ef	71.86 ± 0.61 b	39.09 ± 1.15 d	96.12 ± 1.15 a	39.04 ± 1.31 e	49.57 ± 1.04 b	25.54 ± 0.61 gh
WAG5	17.83 ± 0.64 cd	14.6 ± 0.82 e-g	71.77 ± 0.5 b	39.07 ± 1.43 d	96.03 ± 0.63 a	39.01 ± 1.77 e	49.55 ± 0.81 b	26.16 ± 0.67 gh
WAG6	12.06 ± 0.5 hi	9.45 ± 0.48 j	54.03 ± 1.01 c	25.46 ± 0.07 e	71.96 ± 0.75 d	31.25 ± 2.25 f	32.61 ± 0.1 ef	18.46 ± 0.1 i
WAG7	10.12 ± 0.23 ij	9.15 ± 0.13 j	52.4 ± 1.12 c	26.85 ± 1 e	79.87 ± 0.71 bc	31.22 ± 1.56 f	35.37 ± 0.39 c-e	19.72 ± 0.58 i
WAG8	10.25 ± 0.49 ij	9.55 ± 0.37 j	53.03 ± 1.3 c	27.6 ± 0.55 e	81.79 ± 1.95 b	32.04 ± 2.25 f	37.94 ± 0.37 c	18.17 ± 0.45 i
WAG9	23.06 ± 0.64 a	16.52 ± 0.39 de	76.6 ± 0.45 a	41.64 ± 0.51 d	99.49 ± 0.05 a	40.93 ± 1.61 e	54.61 ± 0.92 a	27.44 ± 0.39 gh
WAG10	10.07 ± 0.52 ij	9.57 ± 0.44 j	53.9 ± 0.55 c	23.89 ± 0.48 ef	75.8 ± 1.15 cd	28.84 ± 1.7 fg	35.59 ± 0.41 cd	17.27 ± 0.44 i
WAG11	24.88 ± 0.64 a	17.2 ± 0.47 d	79.03 ± 0.28 a	41.67 ± 0.64 d	99.73 ± 0.08 a	41.61 ± 0.21 e	56.3 ± 0.39 a	28.12 ± 0.9 g
HSD value ($p \leq 0.05$)	2.3314		4.2110		2.8581		5.9087	

[a] Results represent the mean value ± standard deviation of three replications, Treatment means sharing same letter(s) within the column are statistically non-significant according to Tukey's HSD test at $p \leq 0.05$

In soil results presented in (Table S3) that maximum glyphosate biodegradation was observed at 40˚C that was 89.75 to 97.57 in 100 mg kg^{-1} and 57.59 to 60.47 in 200 mg kg^{-1} by WAG2, WAG4, WAG5, WAG9 and WAG11 bacterial strains. Least degradation was observed in abiotic (without inoculation) condition which was 0.38 and 0.24 at 100 and 200 mg kg^{-1}, respectively. Significant difference was observed between 100 and 200 mg kg^{-1} of GLX at $p<0.05$. The concentration of GLX increasing beyond 100 mg kg^{-1} showed a decline of bacterial growth, which resulted in low rate of biodegradation. *Acetobacter* sp. and *P. fluorescens* were found to thrive best at 7.5 g, with both strains tolerating up to 25 g of GLX (Moneke *et al*., 2010). Despite having additional glucose source, 20 mM GLX greatly reduced the development of *P. oryzihabitans* (Kuklinsky-Sobral *et al*., 2005). *Bacillus cereus* shown to be capable of using the herbicide GLX at an optimal concentration of 6 g (Fan *et al*., 2012b). Several microorganisms have been reported to biodegrade GLX at different temperatures, but 30-40˚C was a preferable temperature. *Ochrobactrum* sp. GDOS (Hadi *et al*., 2013) and *P. putida* (Ouided and Abderrahmane, 2013) showed maximum bacterial growth at 30˚C, recommending vast utilization of GLX by these bacteria. For the meantime, *B. cereus* CB4 revealed optimum biodegradation at 35˚C (Fan *et al*., 2012b).

The biodegradation of pesticides utilized in soil is mainly carried out by microorganisms present in the soil. In four different soils water contents (5%,10%, 15% and 20%) tested here, to check the GLX degradation under biotic and abiotic condition. Results of our experimental study presented in (Table S4) revealed that maximum GLX degradation in control (abiotic) was 27.72 and 12.6 mg kg^{-1} at 100 and 200 mg kg^{-1} respectively, after 28 days of incubation at water content 10% w/v compared to other soil water contents levels. However, at 100 mg kg^{-1} concentration of GLX 96.05 and 96.5 mg kg^{-1} GLX was degraded by WAG2 and WAG9 inoculated treatment in 10% soil water contents after 28 days. Bacterial strains WAG4, WAG5 and WAG11 degraded 84.84 to 91.05 mg kg^{-1} GLX. While in 15% water content soil respective bacterial strains degraded only 71.05 to 79.68 mg kg^{-1} GLX after 28 days. Minimum GLX biodegradation was observed by respective bacterial strains in 20% water contents soil was 55.22 to 59.19 mg kg^{-1}. Significant difference was observed between 100 and 200 mg kg^{-1} of GLX at $p<0.05$.

At 200 mg kg^{-1} of GLX highest degradation of GLX was observed in 10% followed by 15%, 20% and 5% water contents soil. Bacterial strains WAG2 maximum degraded 53.13 mg kg^{-1} GLX in 10% water contents soil after 28 days. However, WAG4, WAG5, WAG9 and WAG11 average degraded 47.60 mg kg^{-1} GLX in 10% water contents soil. Comparatively, highest GLX biodegradation was observed by WAG9 and WAG4 in 10% followed by 15% water contents soil at 100 mg kg^{-1}. While at 200 mg kg^{-1} highest biodegradation was observed by WAG2 in 10% and WAG5 in 15% water content soil. Significant difference was observed between 100 and 200 mg kg^{-1} of GLX at $p<0.05$. Results revealed that low soil moisture contents reduce biodegradation of GLX. The current study lined with (Moorman, 2018; Noshadi and Homaee, 2018). The moisture content of soil is a significant factor that plays an essential part in fumigant diffusion and degradation as well as microbial activity (Mohy-Ud-Din *et al*., 2023). Glyphosate biodegradation in the soil 3.4 times faster when the moisture levels was raised from 5% to 10% in soil. The influence of soil moisture content may be recognized by two aspects. First, the solubilization of GLX in water was reduced at low-moisture content, and the hydrolysis of GLX was obstructed. Thus, the degradation of GLX in the soil reduced with reducing soil moisture content and vice versa, but there were statistically significant differences as the water content was raised from 5% to 10% (Zhang *et al*., 2022). Second, soils with relatively high moisture content can be helpful to the growth of soil microorganisms and maintain greater biodegradation of pesticides. Our experiments suggested that improved soil moisture content from 5% to 10% promoted microbial abundance and its activities, which led to an increase in the rate of GLX biodegradation.

Results from the soil texture to investigate the biodegradation of GLX (clay loam, loam, sandy loam, and sandy clay loam) are summarized in (Table S5). Results revealed that at 100 mg kg^{-1} concentration of GLX average degradation of GLX was carried out by WAG2, WAG4, WAG5, WAG9 and WA11 in sandy loam soil that was 97.11 mg kg^{-1} followed by loam soil 74.10 mg kg^{-1}, sandy clay loam 51.69 mg kg^{-1} and clay loam 21.23 mg kg^{-1} in biotic conditions. While in abiotic conditions maximum degradation of GLX was carried out in clay loam 0.70 mg kg^{-1}, sandy loam 0.59 mg kg^{-1}, loam 0.27 mg kg^{-1} and sandy clay loam 0.14 mg kg^{-1}. At 200 mg kg^{-1} concentration, average GLX degraded by WAG2, WAG4, WAG5, WAG9 and WA11 in loam soil 40.05 mg kg^{-1} followed by sandy loam 39.87 mg kg^{-1}, sandy clay loam 26.50 mg kg^{-1} and clay loam 15.45 mg kg^{-1}. While in abiotic (without inoculation) condition average degradation in all soil texture was only 0.22 mg kg^{-1} after 28 days of incubation.

The breakdown of pesticides utilized in soil is mainly carried out by different microorganisms present in the soil. Therefore, those factors that affect microbial activity in soil also influence the rates of pesticide degradation. Highest GLX biodegradation was observed in sandy loam soil followed by loam, sandy clay loam and clay loam soil in 100 mg kg^{-1} compared to other soil textures. Biodegradation of GLX has been less in 200 mg kg^{-1} as of less availability to isolated bacterial strains. Despite being extremely solubilization in water, GLX moves slowly across the soil profile due to significant adsorption to soil particles (la Cecilia *et al*., 2018). The quantity of clay, iron and aluminum oxides and organic matter present in soil influence GLX adsorption. Strong affinity with soil particles, such as iron and aluminum oxides, may inhibit microbial access to the chemical and hence regulate the pace of GLX breakdown (Hermansen *et al*., 2020). Our findings support previous findings that, in addition to chemical processes, degradation by microorganisms is one of the primary processes of GLX dissipation in soils. Water was used to dissipate GLX in sandy loam soil, making it accessible to isolated bacterial strains that used GLX as a source of carbon and phosphate. While in clay loam soil, various kinds of functional groups, aluminum and iron oxide particles, and adsorption sites are responsible for GLX attachment, making it less accessible to isolated bacterial strains and act as persistent pollutants. Biodegradation was lower at 200 mg kg^{-1} than at 100 mg kg^{-1} GLX concentration. This impact might potentially be attributed to a rather high GLX content (200 mg kg^{-1} of soil), which likely reduced the isolated bacterial activity and/or its breakdown capacity (Coleman *et al*., 2020). These findings are also consistent with prior findings by Foong *et al*. (2020), who discovered that a longer lag phase at higher concentrations may be due to a larger number of microorganisms being required to begin rapid pesticide degradation. However, several research found that greater concentrations of chlorpyrifos and fenitrothion had a detrimental influence on soil microbial activity. Several studies found that the breakdown of hazardous compounds is connected not only to particular microbes but also to the texture of soil. Clay particle, organic matter, pH, and water content are the most essential soil characteristics since they are thought to be the key determinants influencing pesticide adsorption and determining its bioavailability (Rasool *et al*., 2022). Chang *et al*., (2022) found that high chlorpyrifos attraction to soil particles, less bioavailability resulted in poor rate of degradation in clay and silty texture soils. Though, the larger biomass of native microbes in loamy soil with higher organic matter may account for the quicker chlorpyrifos breakdown. This characteristic may be responsible for the quicker breakdown of GLX reported in our research.

Glyphosate-degrading bacteria with plant growth promotion capability: Pearson's correlation matrix heat map showing positive and negative correlations among different parameters in (Fig. 3) (a) liquid medium and (b) in soil medium under incubation trial with 100 and 200 mg kg^{-1} glyphosate. Five bacterial strains were further characterized based on their 16S rRNA gene sequences. WAG2 was identified as *Serratia liquefaciens* with GenBank accession number MW375469, WAG4 as *Klebsiella variicola* with GenBank accession number MW375470, WAG5 as *Enterobacter cloacae* with GenBank accession number MW375471, WAG9 as *Pseudomonas aeruginosa* with GenBank accession number MW375472, and WAG11 as *Enterobacter ludwigii* with GenBank accession number MW375473.

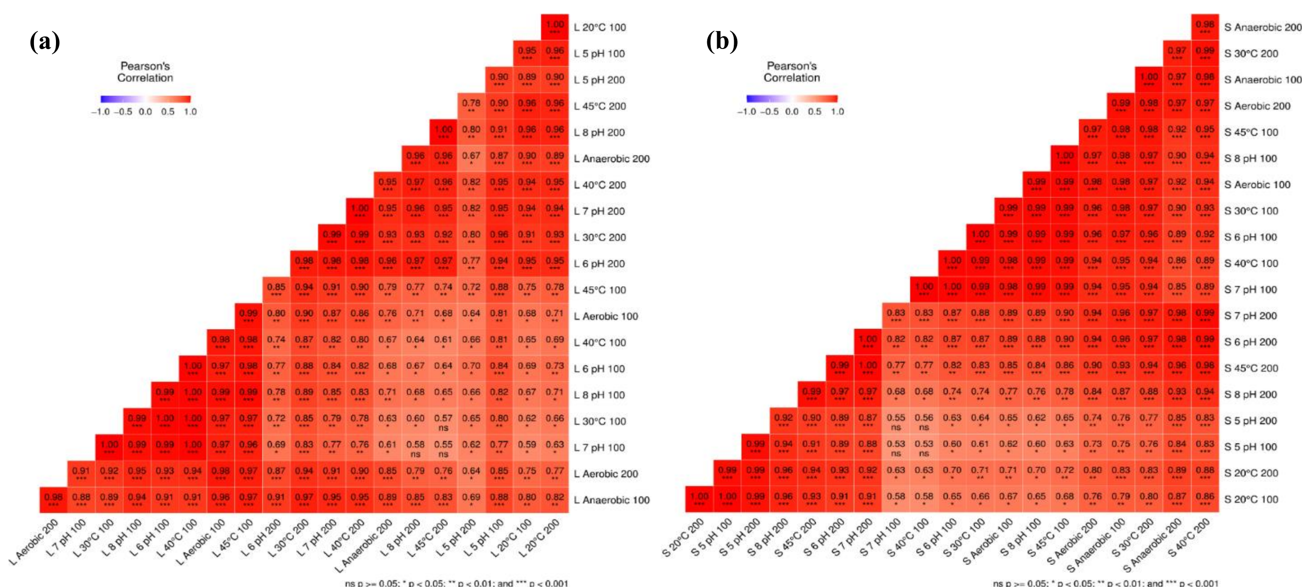

Fig. 3. Pearson's correlation matrix heat map showing correlations among different parameters in (a) liquid medium and (b) in soil under incubation trial with 100 and 200 mg kg^{-1} glyphosate. Results represent the mean value of three replicates.

Conclusions

Our incubation study showed that the five bacterial strains, WAG2 (*Serratia liquefaciens*), WAG4 (*Klebsiella variicola*), WAG45 (*Enterobacter cloacae*), WAG9 (*Pseudomonas aeruginosa*), and WAG11 (*Enterobacter ludwigii*) significantly decline glyphosate concentration in spiked liquid media and soil. These bacterial strains will be deposited in culture collection centers and will be available to other researchers to conduct further tests. Inoculation of bacterial strains with the ability to survive in herbicide spiked liquid media and soil by degrading the glyphosate is a promising technology for agriculture applications. We plan to test these bacterial candidate strains *In vitro* to evaluate their actual impact on different crop plants in the future.

Acknowledgments

We acknowledge the support (to Waqas Mohy-Ud-Din) from the Higher Education Commission (HEC) of Pakistan under the International Research Support Initiative Program (IRSIP) Pakistan. All the authors acknowledge the Soil Microbiology and Biochemistry Laboratory, Institute of Soil and Environmental Science, University of Agriculture Faisalabad, Pakistan, which provided space and all required facilities during this research.

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(Received for publication 14 September 2022)