Effect of Glyphosate Herbicide on the Bacterial Population of Arable Land in Keffi, Nasarawa State, Nigeria

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ABSTRACT

The widespread use of herbicides in agriculture has raised significant concerns regarding their impact on the delicate balance of micro and macro flora and fauna in soil and human health. This study was aimed at effect of glyphosate herbicide on the bacterial population in arable land in Keffi, Topsoil samples were carefully collected from various locations in the Keffi arable land, and the bacteria present were isolated and identified using standard microbiological techniques. The results revealed a stark contrast in bacterial counts between the herbicide-polluted and non-polluted soil samples. In pot A, the bacterial count from the polluted soil was 9.3±1.33 CFU x 10^6, while the non-polluted soil had a count of 14.32±1.02 CFU x 10^6. Similarly, in pot B, the polluted soil had a bacterial count of 8.4±0.99 CFU x 10^6, compared to 13.01±1.54 CFU x 10^6 in the non-polluted soil. In pot C, the polluted soil had a bacterial count of 6.45±1.01 CFU x 10^6, while the non-polluted soil had 14.01±1.88 CFU x 10^6. The bacteria isolated from the different pots were also diverse in their composition. Pot A was dominated by *Pseudomonas* sp., *Priestia* sp., and *Bacillus* sp. (33.3% each), while pot B was dominated by *Pseudomonas* simiae (66.6%) and *Proteus* sp. and *Bacillus magaterium* (33.3% each). Pot C, was dominated by *Bacillus* sp. (66.6%) and *Acinetobacter beijerinckii* and *Citrobacter* sp. (33.3% each). Interestingly, several of the isolated bacteria, including *Pseudomonas* sp., *Proteus* sp and *Acinetobacter beijerinckii*, were able to survive and grow in the presence of the glyphosate herbicide. *Pseudomonas* sp. isolated from pot A1a exhibited the highest utilization of the herbicide, at 30°C (2.19±0.26 mg/ml) and the lowest at 26°C (1.23±0.11 mg/ml). Similar trends were observed for other isolates, with *Proteus* sp., *Priestia* sp., and *Bacillus magaterium* showing the highest herbicide utilization at 35°C. *Pseudomonas simiae* isolated from pot A1a demonstrated the highest utilization at pH 7.0 (3.54±0.32 mg/ml), and the lowest at pH 5.0 (1.68±0.57 mg/ml). Additionally, the same isolate showed the highest herbicide utilization after 4 and 5 weeks of incubation (2.08±0.02 mg/ml), and the lowest in week 1 (1.18±0.07 mg/ml). The findings of this comprehensive study clearly demonstrate the detrimental impact of glyphosate herbicide on the bacterial population in the arable land of Keffi, Nasarawa State.

Keywords: herbicide, utilization, polluted soil, glyphosate and isolates

INTRODUCTION

Soil is known as a complex and dynamic biological system, hosting a wide diversity of living organisms, accomplishing various functions such as biogeochemical cycles [1], supporting numerous soil ecosystem services such as primary production and water. A single gram of soil shelters several tens of thousands different species of microorganisms and billions of bacteria [2]. Microorganisms are not only numerous in soils, they are active where they develop keystone functions for the ecosystem such as N-cycling [3].

Herbicides or “weed killers” are a group of chemicals known as pesticides, which prevent, inhibit, destroy, repel or mitigate or kill weeds and other undesirable plants. Selective herbicides kill specific targets, while leaving the desired crop relatively unharmed. Other familiar pesticides are insecticides, rodenticides and fungicides. There are many different types of herbicides, all of which can be dangerous to humans or the environment if used irresponsibly [4]. Consequently, herbicides as hazardous materials have been increasing in

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recent years. Large quantities of herbicides accumulate in the top layer of soil, leading to alterations in soil biota [5,6].

The use of herbicides in agriculture has over the years contributed tremendously to both food and cash crop production all over the world of which Nigeria is not an exception. But one of the challenges undermining the farming business [7], has been the invasion of many common weed species due to favourable environmental conditions such as abundance of rainfall, adequate sunlight, fertile soil etc. in Nigeria. As a result, manufacturers have adopted flooding the agrochemical market with all kinds of herbicides that are meant for the elimination of different kinds of weeds at different stages of their growth [8]. Perhaps, the efficacy of these herbicides in controlling the target weeds has resulted in the application of these chemicals by most farmers. The soil serves as the repository for all agricultural contaminants, function as a major habitat for most microbial communities such as soil bacteria, fungi and actinomycetes whose activities influences the soil fertility [9], through organic material degradation, organic matter decomposition and nutrient cycling [10,11]. Nonetheless, over application of these chemicals inhibit some of these natural processes, and decreases the performance of the non-target organisms [12]. However, some soil organisms use these herbicides in the process of degradation as carbon and energy source for their metabolic activities. Hence, the need to study the effects herbicides which are commonly used in Keffi in order to assess their inhibitory effects on some of the beneficial microorganisms in the soil

METHODS

Study Area

The study was carried out in Keffi. located on longitude 7° 50`$^{E}$ and latitude $8^{\circ}$ 3`^{N}, North West of Lafia (The Capital of Nasarawa State, Nigeria), and is situated on an altitude of 850M above sea level. Keffi, though in Nasarawa State is about 68km from Abuja, the Federal Capital of Nigeria [13].

Collection and Processing of Samples

The topsoil (up to 5 cm depth) sample was collected from arable land in three locations in Keffi with no prior herbicide treatment. The soil samples were collected in triplicates at each of the sampling sites using a sterile spoon. The samples were mixed thoroughly, and portions were taken in a clean sealable sterile polythene bags for laboratory analyses. The samples were sieved using a 2.0mm mesh size to remove stones and plant debris.

Determination of Bacterial Load from herbicide polluted soil and non-polluted soil

Fifty millilitre of the herbicide was mixed with 100g of the soil sample in different pots labelled pot A, pot B and pot C and incubated at ambient temperature for 7days. The enumeration of the bacteria population was done using Pour Plate method. One (1.0) gram of the soil sample from the pot was weighed using weighing balance and was suspended in 9ml of sterile water. It was properly mixed and a 10-fold serial dilution was carried out. 1ml of the soil suspension was transferred into test tube containing 9ml of sterilized water and other 1ml from the 1st test tube was transferred to 2nd test tube containing 9ml of sterilized water and this step was repeated to 10th test tube. The diluted samples from 10^{-7} were plated on sterile prepared nutrient agar and incubated at 37^{o}C for 24 hours. The isolates were sub-cultured on nutrient agar which were grown and stored on agar slants in the Refrigerator at 4°C as stock culture for further identification

Characterization and Identification of Bacterial Isolates

Characterization of Isolates

Characterization of the bacteria was done based on morphological characteristics and biochemical tests carried out on the isolates. Morphological characteristics observed for each bacterial colony after 24hours of growth include colony appearance; shape, elevation, edge, optical characteristics, consistency, colony surface and pigmentation. Identification of the isolates was done by comparing the characteristics of the isolates with those of known taxa using scheme of Bergey's Manual of Determinative Bacteriology 9th Edition.

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Biochemical Characterization

In order to identify the purified cultures tentatively, biochemical tests were performed as described below.

Utilization/Degradation of Herbicide

This was carried out by adopting the method of Tanney *et al.*, [14]. Degradation was measured by checking cell growth through optical density at 3days interval. Turbidity was measured using spectrophotometer.

Determination of the effect of Temperature, pH and Days on biodegradation

Biodegradation experiments were carried out at three different temperatures (26^{o}C, 30^{o}C and 35^{o}C), pH (pH 5.0, pH 5.5, pH 6.0, pH 6.5 and pH 7.0) and time (1-5weeks) using the methods of Tanney *et al.*, [14].

Determination of the Effect of Temperature

Experiment was carried out at various temperatures (26℃, 30℃, 35℃) in order to determine the effect of temperature on biodegradation of herbicides by bacteria. The isolates were Inoculated in mineral salt medium broth containing Glyphosate (Round up) herbicide of 50 ppm ($NaNO_3$ 3.0 g, K_2HPO_4 1.0g, KCl 0.5g, $MgSO_4$ 0.1 g, $FeSO_4$.2H2O 0.1g, Sucrose 30g) were incubated for 15days [15].

Determination of the Effect of pH

In order to determine the effect of pH on biodegration potential of bacteria, the isolates were inoculated in mineral salt medium broth containing Glyphosate herbicide of 50 ppm ($NaNO_3$ 3.0 g, K_2HPO_4 1.0g, KCl 0.5g, $MgSO_4$ 0.1 g, $FeSO_4.2H_2O$ 0.1g, Sucrose 30g) the pH were adjusted to pH 5.0, pH 5.5, pH 6.0, pH 6.5 and pH 7.0 and were incubated for 15days [15].

Determination of the Effect of Time (weeks)

Effect of time (weeks) on herbicide biodegradation was carried out as described by Kumar *et al*, [15]. The isolates were inoculated in mineral salt medium broth containing Glyphosate (Round up) herbicide of 50 ppm ($NaNO_3$ 3.0 g, K_2HPO_4 1.0g, KCl 0.5g, $MgSO_4$ 0.1g, $FeSO_4.2H_2O$ 0.1g, Sucrose 30g) and were incubated for different time (weeks) ranging from 1 to 5 weeks.

Determination of Herbicide Utilization by different bacteria isolated

Herbicides utilization was carried out using a method described by Kumar *et al*, [15]. After incubation, the broth media was centrifuged at 1000rpm for 2mins. The residues of the herbicide were analyzed by UV-visible Spectrophotometer**.**

Molecular Identification of Bacterial Isolates Using 16S rRNA

DNA Extraction

The DNA extraction of bacterial species was carried out as described by Makut *et al.* [16]. Two hundred and fifty millilitres of pure colonies of the suspected organism, dissolved in 10ml of Luria-Bertani Broth, was added to a Bashing Bead Lysis Tube (0.1 and 0.5) and 750 µl Lysis Solution was added to the tube. A bead beater fitted with a 2 ml tube holder assembly was secured and processed at a maximum speed for 5 minutes.

The Bashing Bead Lysis Tube was centrifuged in a microcentrifuge at 10,000 x g for 1 minute. 400µl supernatant was transferred to a Zymo-Spin IV Spin Filter in a collection tube. Eight hundred microliters of the mixture was transferred to a Zymo-Spin IIC column in a collection tube and was centrifuged at 10,000 x g for 1 minute. The flow through from the collection tube was discarded. 200 µl DNA Pre-Wash Buffer was added to the Zymo-Spin IIC Column in a new collection tube and was centrifuged at 10,000 x g for 1 minute.

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Five hundred microliters DNA Wash Buffer was added to the Zymo-Spin IIC Column and was centrifuged at 10,000 x g for 1 minute 800 µl of the mixture was transferred to a Zymo-Spin IIC Column in a collection tube and was centrifuged at 10,000 x g for 1 minute. The flow through was discarded from the collection tube. 1, 200 µl of DNA Binding Buffer was added to the filtrate in the collection tube.

The Zymo-Spin IIC Column was transferred to a clean 1.5 ml microcentrifuge tube and100 µl DNA Elution Buffer was added directly to the column matrix and centrifuged at 10,000 rmp for 30 seconds to elute the DNA.

The base of the Zymo-Spin IV-HRC Spin Filter was snapped off and placed into a collection tube and was centrifuged at 8,000 rmp for 3 minutes. The eluted DNA was transferred to a prepared Zymo-Spin IV-HRC Spin Filter in a clean 1.5 ml microcentrifuge tube and was centrifuged at 8,000 rmp for 1 minute. Extracted DNA was stored at -20ºC until use.

The DNA Amplification

DNA amplification was conducted using a forward primer and a reverse primer specific for the genus bacteria specific for the 16S rRNA. The primer amplified a product of 1078 base pairs (bp) in the 16SrRNA genome. The PCR was performed in a 10 µl reaction volume containing 3.5 µl of 2× Master Mix from Promega; 3.0 of 25ng/µl DNA and 2.0µl of water and 0.5 µl of each oligonucleotide primer (5pMol forward primer and 5pMol reverse primer Inqaba Biotechnical Industries (Pty) Ltd, Pretoria, South Africa).

The mixtures were subjected to initial denaturation at 94 ºC for 5 minutes, followed by 36 cycles of amplification involving denaturation at 94 ºC for 30 seconds, primer annealing at 56 ºC for 30 seconds, and primer extension at 72 ºC for 45 sec; a final primer extension at 72 ºC for 7 minutes, using a DNA thermal cycler (GeneAmp PCR system 9700, Applied Biosystems, South Africa).

PCR Product Purification

The PCR product purification was carried out using a method described by Makut *et al.* [16]. In this method, 20µl of absolute ethanol was added to the PCR product and then incubated at room temperature (±) for 15minutes and spun at 10,000rpm for 15minutes. The supernatant was decanted and spun at 10, 000 rpm for 15 minutes. Twenty microliter (20µl) of 70% ethanol was added and again the supernatant was decanted and air-dried. About 10µl of ultrapure water ordered from Oxford, the United Kingdom was added. Amplicon on 1.5% agarose was checked for**.** The product from the purification process was loaded on the 3130xl genetic analyzer from Applied Biosystems to give the sequences.

Sequencing Analysis

PCR amplification DNA were sent to Inqaba Biotechnical Industries (Pty) Ltd, Pretoria, South Africa for sequencing using the primer pair as shown in Appendix 1. Sequencing data obtained were assembled and edited to a total length of 1078 bp using free sequencing software (BioEdit) was to view and analyse the data. BLAST (Basic Local Alignment Search Tool) searches of the sequences were conducted using the techniques developed by the National Centre for Bioinformatics, Islamabad, Pakistan, to determine the similarity between sequencing data obtained from local strains and those available in GenBank. Data were recorded as percentage similarity to related species.

Statistical Analysis

Data collected from the study was analyzed using general descriptive statistics, one Way Analysis of Variance (ANOVA) at 95% probability level of significance. If significant differences were found, Duncan's multiple range tests was used to compare the different experimental groups. Computer software such as Microsoft Excel was used for the statistical analyses (IBM Corp. 2021).

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RESULTS

Bacterial Count from Polluted and Non-polluted Soil

The bacterial count from polluted and Non-polluted Soil after one month is shown in Table 1. The bacterial count from polluted soil pot A was 9.3±1.33 cfu x10^6 while from Non-polluted soil was 14.32± 1.02 cfu x10^6. The bacterial count from pot B polluted soil was 8.4± 0.99 cfu x10^6 and Non-polluted 13.01±1.54 cfu x10^6 and from pot C the bacterial count from polluted soil was 6.45±1.01 and from non-polluted was 14.01±1.88 cfu x10^6.

Cultural, Morphological and Biochemical Characteristics of bacterial Isolates from Polluted and Non polluted soil

The cultural, morphological and biochemical characteristics of the bacterial isolates are as shown in Table 2 and 3. where Milksh, circular, coarse, flat, convex, entire and opaque colonies, Gram negative, catalase positive, oxidase negative, indole negative, nitrate positive were suspected to be *Bacillus subtilis,* smooth none elevated colonies with green pigment on NA, gram negative, catalase negative, oxidase negative, indole negative, nitrate positive were suspected to be *Pseudomonas flourescens.* Yellowish pigment smooth edge and large colony on NA, gram negative, catalase negative, oxidase negative, indole positive, nitrate positive were suspected to be *Gluconbacter* sp. Large, circular, regular and milky white on NA Gram positive, catalase negative, oxidase negative, indole positive, nitrate negative were suspected to be *Acetobacter* sp. Colourless colonies, flat, swarm on MaC and NA Gram negative, catalase negative, oxidase negative, indole positive, nitrate positive were suspected to be *Citrobacter* sp

Table 1: Bacterial Count from polluted and Non-polluted Soil after one month

Sample	**Viable Count (cfu x10^6) Polluted**	**Non-polluted**
Pot A	9.3±1.33	14.32± 1.02
Pot B	8.4± 0.99	13.01±1.54
Pot C	6.45±1.01	14.01±1.88

Table 2 Cultural, Morphological and Biochemical Characteristics of bacterial isolates from Non-polluted Soil

Sample	Cultural morphology	GR	Biochemical Characteristics							Inference
			Cat	In	Ox	Nit	MR	Cit	Ur	
Pot A 1a	Greenish on NA	-	+	-	+	+	-	-	+	*Pseudomonas* sp
1b	Yellowish smooth edge and large colony	-	-	+	-	+	-	+	-	*Gluconbacter* sp
2a	Brown mucoid smooth on Mac Agar	-	+	+	-	+	+	-	+	*Proteus* sp
2b	Creamy, bulk dried and nono smooth edge	+	+	-	-	+	-	+	-	*Bacillus* sp
2c	Creamy, raised colony	+	-	+	+	+	+	-	+	*Enterococcus* sp

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Pot B 1a	Large, circular, regular and milky white on NA	-	+	-	-	-	-	+	-	*Acinetobacter* sp
1b	Brown mucoid smooth on Mac Agar	-	+	+	-	+	+	-	+	*Proteus* sp
2a	colonies were Colourless, flat and swarm on MCA and NA	-	-	+	-	+	+	-	+	*Entrobacter* sp
Pot c 1a	Greenish on NA	-	+	-	+	+	-	-	+	*Pseudomonas* sp
2b	Creamy, raised colony on NA and purple on MCA	+	-	+	+	+	+	-	+	*Enterococcus* sp
2c	Colonies were Colourless, flat and swarm on NA	-	-	+	-	+	+	-	+	*Entrobacter* sp

*GR=Gram Reaction; Cat=Catalase; In=Indole; Ox=Oxidase; Nit=Nitrate; MR=Methy Red;

Cit=Citrate; Ur=Urease

Table 3 Cultural, Morphological and Biochemical Characteristics of bacterial isolates from polluted Soil

Isolates	Cultural morphology	G/R	Biochemical Characteristics							Inference
			Cat	In	Ox	Nit	MR	Cit	Ur	
Plot A 1a	Greenish colonies on NA	-	+	-	+	+	-	-	+	*Pseudomonas* sp
2b	Creamy, bulk dried and nono smooth edge	+	+	-	-	+	-	+	-	*Bacillus* sp
2d	Greenish colonies on NA	-	+	-	+	+	-	-	+	*Pseudomonas* sp
Plot B 1a	Large, circular, regular and milky white on NA	-	+	-	-	-	-	+	-	*Acinetobacter* sp
2b	Brown mucoid smooth on Mac Agar	-	+	+	-	+	+	-	+	*Proteus* sp
Plot c 1a	Greenish colonies on NA	-	+	-	+	+	-	-	+	*Pseudomonas* sp
	Colonies are Colourless, flat and swarm MCA and NA	-	-	+	-	+	+	-	+	*Citrobacter* sp

*GR=Gram Reaction; Cat=Catalase; In=Indole; Ox=Oxidase; Nit=Nitrate; MR=Methy Red;

Cit=Citrate; Ur=Urease

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Occurrence of Bacteria in soil Samples

Occurrence of different bacteria in polluted Soil with glyphosate (Roundup) herbicide is as given in Table 4. The bacteria from Pot A were *Pseudomonas* sp, *Priestia* sp and *Bacillus* sp (33.3 %). From Pot B different bacteria isolated were *Pseudomonas* sp (66.6 %), *Proteus* sp and *Bacillus* sp (33.3 %) and from Pot C were *Bacillus* sp (66.6 %), *Acinetobacter* sp, *Pseudomonas* sp and *Citrobacter* sp (33.3 %) respectively.

Screening Survival in Glyphosate (Roundup) Herbicide Broth

Table 5 shows bacterial survival in glyphosate (Roundup) herbicide broth. *Pseudomonas* sp isolated from Pot A1a and Pot C 2b, *Proteus* sp isolated from Pot B 3a, *Priestia* sp isolated from Pot A 3c, *Bacillus* sp isolated from Pot B 3a and Pot C 2c and *Acinetobacter* sp Pot C 3b were able to grow in herbicides broth

Utilization of Glyphosate (Roundup) Herbicides at Different Temperature

Bacterial utilization of glyphosate (Roundup) herbicides at different temperature is as shown in Table 6. *Pseudomonas* sp isolated from Pot A 1a was able to utilize highest herbicide at 30 ℃ (2.19±0.26 mg/ml) followed by 35 ℃ (2.06±0.64 mg/ml) and the lowest was at 26 ℃ (1.23±01.1mg/ml). *Pseudomonas* sp isolated from Pot C 2b was able to utilize highest herbicide at 30 ℃ (2.15±0.08mg/ml) followed by 35 ℃ (2.01±0.23mg/ml) and the lowest was at (1.14±0.29 mg/ml). *Proteus* sp isolated from Pot B 3a had the highest utilization at 35 ℃ (1.97±0.05 mg/ml) followed by 30 ℃ (1.92±0.16 mgml) and the least was at 26 ℃ (1.55±0.15 mg/ml). The highest utilization observed from *Priestia* sp isolated from Pot A 3c was at 35 ℃ (2.12±0.19mg/ml) followed by 30 ℃ (1.92±0.16mg/ml) and 26 ℃ (1.55±0.15 mg/ml). From *Bacillus* sp isolated from Pot B 3a the highest utilization was recorded at 35 ℃ (2.00±0.03 mg/ml) followed by 30 ℃ (1.80±0.05 mg/ml) and lowest at 26 ℃ (1.48±0.24mg/ml). *Bacillus* sp isolated from Pot C 2c recorded highest utilization at 35 ℃ (2.02±0.57 mg/ml) followed by 30 ℃ (1.62±0.08 mg/ml) and lowest was recorded at 26 ℃ (1.02±0.86 mg/ml). *Acinetobacter* sp isolated from Pot C 3b had highest utilization at 35 ℃ (1.86±0.28 mg/ml) followed by 30 ℃ (1.27±0.35 mg/ml) and the lowest was at 26 ℃ (1.17±0.35 mg/ml) respectively.

Utilization of Glyphosate (Roundup) Herbicides at Different pH

The bacterial utilization of glyphosate (Roundup) herbicide at different pH is as shown in Table 7. *Pseudomonas* sp isolated from Pot A 1a was able to utilize highest herbicide at pH 7.0 (3.54±0.32mg/ml) followed by pH 6.5 (3.08±0.26mg/ml), pH 6.0 (2.04±0.02mg/ml) and the lowest was at pH5.0 (1.68±0.57/ml). *Pseudomonas* sp isolated from Pot C 2b utilized highest herbicide at pH 7.0 (2.89±0.82 mg/ml) followed by pH 6.5 (2.15±0.28 mg/ml), pH 6.0 (1.99±0.85 mg/ml), pH 5.5 (1.97±0.01mg/ml) and the lowest was at pH5.0 (1.74±0.91mg/ml). *Proteus* sp isolated from Pot B 3a had the highest utilization at pH 7.0 (2.09±0.19 mg/ml) followed by pH 6.5 (1.93±0.15 mg/ml), pH 5.5 (1.71±0.15 mg/ml) and the least was at pH5.0 (1.06±0.80 mg/ml). Highest utilization recorded from *Priestia* sp isolated from Pot A 3c was at pH 7.0 (3.34±0.02 mg/ml) followed by pH 6.5 (3.10±0.21 mg/ml), pH 6.0 (2.84±0.22 mg/ml), pH 5.5 (2.14±0.02 mg/ml) and the lowest was at pH5.0 (1.82±0.16mg/ml). From *Bacillus* sp isolated from Pot B 3a the highest utilization was recorded from pH 7.0 (3.61±0.85 mg/ml) followed by pH 6.5 (3.00±0.63 mg/ml), pH 6.0 (2.90±0.85 mg/ml) pH 5.5 (2.29±0.19 mg/ml) and lowest from pH5.0 (2.69±1.01 mg/ml). *Bacillus* sp isolated from Pot C 2c recorded highest utilization at pH7.0 (2.89±0.29 mg/ml) followed by pH6.5 (2.78±0.26 mg/ml), pH 6.0 (2.59±0.09 mg/ml), pH 5.5 (2.29±0.19 mg/ml) and lowest was recorded at pH5.0 (1.27±0.11 mg/ml). *Acinetobacter* sp isolated from Pot C 3b had highest utilization at pH 7.0 (2.62±0.14mg/ml) followed by at pH 6.5 (2.03±0.55mg/ml), pH 6.0 (1.82±0.24mgml) and the lowest at pH 5.0 (1.43±0.18mg/ml) respectively.

Utilization of Glyphosate (Roundup) Herbicide at Different Week

The bacterial utilization of herbicide in different weeks is shown in Table 8. *Pseudomonas* sp isolated from Pot A 1a recorded highest herbicide utilization after 4 and 5 Weeks (2.08±0.02 mg/ml) followed by Week 3 (3.08±0.26mg/ml), Week 2 (1.89±0.27mg/ml) and the lowest was after Week 1 (1.18±0.07 mg/ml). *Pseudomonas* sp isolated from Pot C 2b recorded highest utilization of herbicide after Week 3 (3.11±0.85mg/ml) followed by 4 Weeks (2.95±0.08 mg/ml), Week 5 (2.93±0.05 mg/ml), Week 2 (2.92±0.01

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mg/ml) and the lowest was after Week 1 (2.84±0.11 mg/ml). *Proteus* sp isolated from Pot B 3a had the highest utilization after week 3 (3.59±0.19 mg/ml) followed by week 4 and 5 (2.14±0.55 mg/ml), week 2 (2.11±0.05 mg/ml) and the least was after Week 1 (2.00±0.80 mg/ml). Highest utilization was observed from *Priestia* sp isolated from Pot A 3c after week 4 and 5 (2.10±0.31mg/ml) followed by week 3 (2.04±0.02 mg/ml), week 2 (1.98±0.02 mg/ml) and the lowest was after Week 1 (1.82±0.16 mg/ml). From *Bacillus* sp isolated from Pot B 3a the highest utilization was recorded from week 5 (3.90±0.85 mg/ml) followed by week 4 (3.88±0.62 mg/ml), week 3 (3.61±0.85 mg/ml), week 2 (1.89±0.41 mg/ml) and lowest from week 1 (1.89±0.41 mg/ml). *Bacillus* sp isolated from Pot C 2c recorded highest utilization after week 5 (3.68±0.19 mg/ml) followed by week 4 (3.67±0.26 mg/ml), week 3 recorded (3.59±0.19 mg/ml), week 2 (2.99±0.19 mg/ml) and lowest was recorded after week 1 (2.17±0.19 mg/ml). *Acinetobacter* sp isolated from Pot C 3b had highest utilization after week 4 and 5 (2.73±0.55 mg/ml) followed by week 3 (2.62±0.24 mg/ml), week 2 recorded (2.62±0.24 mg/ml) and the lowest after week 1 (1.13±0.08 mg/ml) respectively

Table 4: Occurrence of different Bacteria in polluted Soil with Glyphosate (Roundup®) Herbicide

Bacteria	**No. Sampled**	**Pot A No. (%)**	**Pot B No. (%)**	**Pot C No. (%)**
Pseudomonas sp	3	1(33.3)	2(66.6)	1(33.3)
Proteus sp	3	0(0.0)	1(33.3)	0(0.0)
Priestia sp	3	1 (33.3)	0 (0.0)	0(0.0)
Bacillus sp	3	1(33.3)	1(33.3)	2(66.6)
Acinetobacter sp	3	0(0.0)	0(0.0)	1(33.3)
Citrobacter sp	3	0(0.0)	0(0.0)	1(33.3)

Table 5: Screening of bacterial Isolates for Survival in Glyphosate (Roundup®) Herbicide Broth

Bacteria	**Lab Code**	**Utilization**
Pseudomonas sp	Pot A 1a	+
Pseudomonas sp	Pot C 2b	+
Pseudomonas sp	Pot C 4a	-
Proteus sp	Pot A 1b	-
Proteus sp	Pot A 2a	-
Proteus sp	Pot B 3a	+
Priestia sp	Pot A 3c	+
Bacillus sp	Pot B 3a	+
Bacillus sp	Pot B 1a	-
Bacillus sp	Pot C 2c	+
Gluconbacter sp	Pot A 1b	-

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Acinetobacter sp	Pot C 3b	+
Entrobacter sp	Pot B 2c	-
Citrobacter sp	Pot C 1c	-

Keys: + = Positive; - = Negative

Table 6: Bacterial Utilization of Glyphosate (Roundup®) Herbicide at different Temperature

			Amount of herbicide utilization (mean ± S.D) (mg/ml)		
Lab Code	**Isolates**	**Control (mg/ml)**	**26 ℃**	**30 ℃**	**35 ℃**
Pot A 1a	*Pseudomonas* sp	5.01±0.07	1.23±01.1	2.19±0.26	2.06±0.64
Pot C 2b	*Pseudomonas* sp	5.01±0.07	1.14±0.29	2.15±0.08	2.01±0.23
Pot B 3a	*Proteus* sp	5.01±0.07	0.53±0.86	1.92±0.16	1.97±0.05
Pot A 3c	*Priestia* sp	5.01±0.07	1.55±0.15	1.94±0.34	2.12±0.19
Pot B 3a	*Bacillus* sp	5.01±0.07	1.48±0.24	1.80±0.05	2.00±0.03
Pot C 2c	*Bacillus* sp	5.01±0.07	1.02±0.86	1.62±0.08	2.02±0.57
Pot C 3b	*Acinetobacter* sp	5.01±0.07	1.17±0.35	1.27±0.35	1.86±0.28

Table 7 Bacterial Utilization of Glyphosate (Roundup) Herbicide at Different pH Level

		Control (mg/ml)	**Amount of Herbicide Utilization (Mean ± S.D) (mg/ml)**				
Lab Code	**Bacteria**		**pH5.0**	**pH 5.5**	**pH 6.0**	**pH 6.5**	**pH 7.0**
Pot A 1a	*Pseudomonas* sp	5.01±0.07	1.68±0.57	1.79±0.27	2.04±0.02	3.08±0.26	3.54±0.32
Pot C 2b	*Pseudomonas* sp	5.01±0.07	1.74±0.91	1.97±0.01	1.99±0.85	2.15±0.28	2.89±0.82
Pot B 3a	*Proteus* sp	5.01±0.07	1.06±0.80	1.71±0.15	1.89±0.19	1.93±0.15	2.09±0.19
Pot A 3c	*Priestia* sp	5.01±0.07	1.82±0.16	2.14±0.02	2.84±0.22	3.10±0.21	3.34±0.02
Pot B 3a	*Bacillus* sp	5.01±0.07	2.69±1.01	2.81±0.05	2.90±0.85	3.00±0.63	3.61±0.85
Pot C 2c	*Bacillus* sp	5.01±0.07	1.27±0.11	2.29±0.19	2.59±0.09	2.78±0.26	2.89±0.29
Pot C 3b	*Acinetobacter* sp	5.01±0.07	1.43±0.18	1.69±0.16	1.82±0.24	2.03±0.55	2.62±0.14

Table 8: Bacterial Utilization of Glyphosate (Round up) Herbicide in Different Time (Weeks)

		Control (Mg/ml)	**Amount of Herbicide Utilization (Mean ± S.D) (mg/ml)**				
Lab Code	**Bacteria**		**1 Week**	**2 Weeks**	**3 Weeks**	**4 Weeks**	**5 Weeks**
Pot A 1a	*Pseudomonas* sp	5.01±0.07	1.18±0.07	1.89±0.27	2.04±0.02	2.08±0.16	2.08±0.02
Pot C 2b	*Pseudomonas* sp	5.01±0.07	2.84±0.11	2.92±0.01	3.11±0.85	2.95±0.08	2.93±0.05

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Pot B 3a	*Proteus* sp	5.01±0.07	2.00±0.80	2.11±0.05	3.59±0.19	2.14±0.55	2.14±0.09
Pot A 3c	*Priestia* sp	5.01±0.07	1.82±0.16	1.98±0.02	2.04±0.02	2.10±0.31	2.10±0.12
Pot B 3a	*Bacillus* sp	5.01±0.07	1.89±0.41	2.01±0.85	3.61±0.85	3.88±0.62	3.90±0.85
Pot C 2c	*Bacillus* sp	5.01±0.07	2.17±0.19	2.99±0.19	3.59±0.19	3.67±0.26	3.68±0.19
Pot C 3b	*Acinetobacter* sp	5.01±0.07	1.13±0.08	2.09±0.19	2.62±0.24	2.73±0.55	2.73±0.14

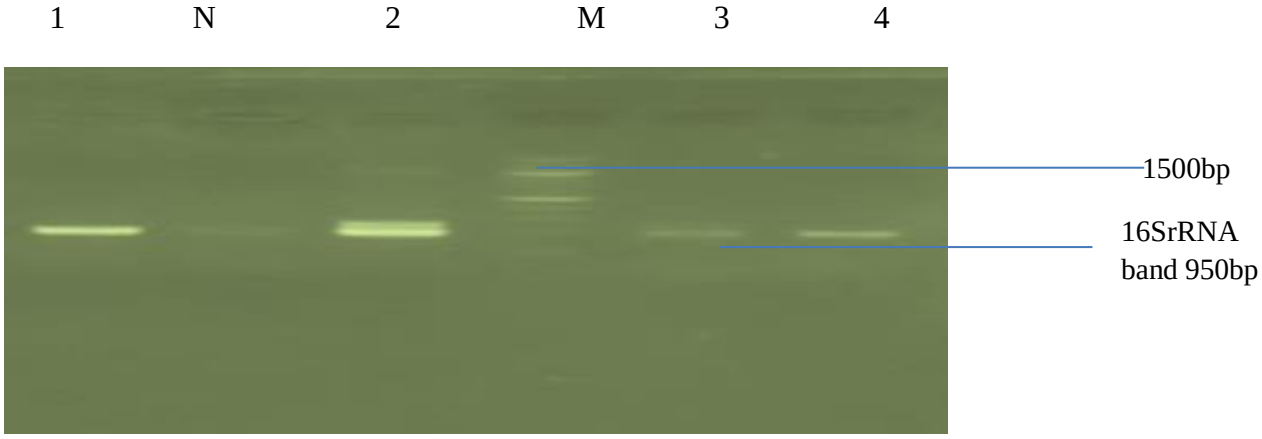

Plate 1: Agarose gel electrophoresis of the 16S rRNA gene of bacteria isolated from

Polluted soil with glyphosate (Roundup) herbicide. Lanes 1-4 represent the *Pseudomonas simiae, Bacillus magaterium*, *Pseudomonas rhodesiae*, and *Acinetobacter beijerinckii* 16SrRNA gene bands of 1000bp. Lane N represents negative control Lane M represents the 1500bp molecular ladder

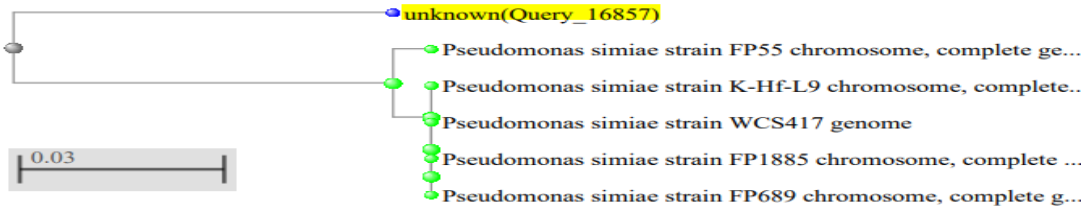

Figure 4.1: Phylogenetic tree showing the evolutionary distance between *Pseudomonas simiae* isolated

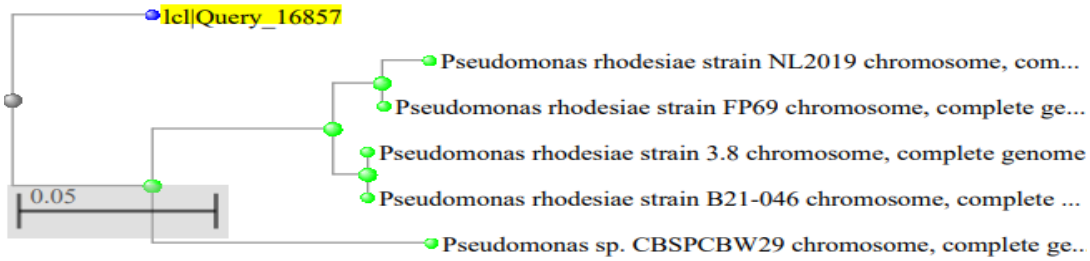

Figure 2: Phylogenetic tree showing the evolutionary distance between *Pseudomonas rhodesiae* isolated

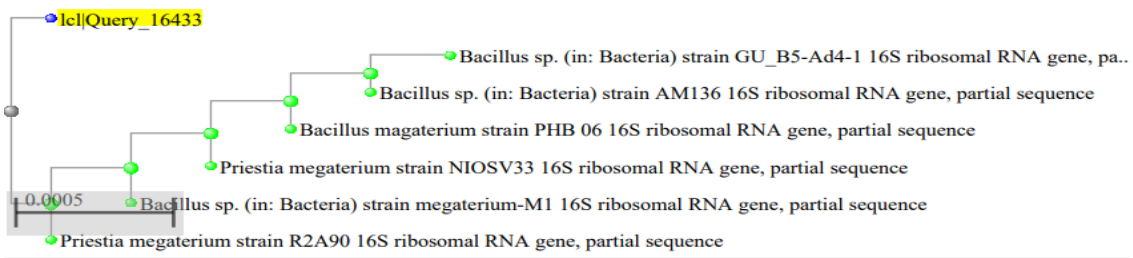

Figure 3: Phylogenetic tree showing the evolutionary distance between *Bacillus magaterium* isolated

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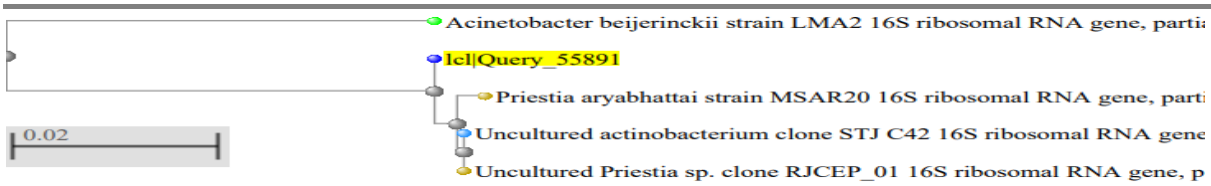

Figure 4: Phylogenetic tree showing the evolutionary distance between *Acinetobacter beijerinckii* isolated

DISCUSSION

Microorganisms play important role in maintaining soil structure and fertility. Herbicides applied at higher dose are persistent in the soil and they have negative effect on soil microorganism. In this study, it was observed that there was reduction in bacterial population in soil polluted with glyphosate (Roundup) herbicide when compared with soil that was not polluted with herbicides. This shows that chemical composition of the herbicide affects the microorganisms inhibiting their growth or it may be due to the concentration of the herbicide used. The bacteria isolated from the polluted soil were *Pseudomonas* sp, *Priestia* sp and *Bacillus* sp, *Proteus* sp, *Acinetobacter* sp and *Citrobacter* sp. These are mostly bacteria that are predominantly found in the soil and are known to fix nitrogen, phosphate and other chemicals in the ecosystem. This finding is similar to work reported by Sviridov *et al*., [17] and Kremer *et al.* [18].

The different bacteria isolated from herbicide polluted soil showed the ability to use herbicide as carbon source when grown in herbicide incorporated media. Temperature showed to be an important factor in bacterial growth and metabolism. In this study it was observed that the different bacteria isolated utilized the herbicide maximally at 30℃ this may be due to the environmental temperature of the study location which always range between 28℃ and 32℃ every day, which have made the bacteria well adapt to the temperature as their optimum temperature for growth and metabolic activities. This is in agreement with the study reported by Mendes *et al.* [19] who observed 32℃ as optimum temperature in the study of the effect of temperature on degradation of herbicide by bacteria isolated from rice farm.

Also, in this study it was observed that pH of the soil played important role in the degradation of the herbicide. The acidity or alkaline level of any environment affects the microbial population of that environment. It was found that when the pH of the soil was kept at 7.0, the bacteria were able to utilize the herbicide more than when it was too acidic pH 5.0. This implies that the application of herbicides tends to change the pH of the soil and caused the depletion of the population of microorganism in the soil and this may affect circulation of nutrients in the soil. This is in line with the study reported by Gimsing *et al.* [20] and Ermakova *et al.* [21] who studied the Kinetics growth of bacteria in soil polluted with herbicide. The finding from this study on the effect of pH on utilization of herbicide also, indicates that these bacteria isolated from polluted soil with herbicide have found a way of adaption to such environment when the pH of the soil was adjusted because of the application of herbicide, as they were able to utilize the herbicide at a lower pH.

The finding in this study also showed that it took the different bacteria isolated from herbicide polluted soil some weeks or months to degrade the herbicide.

This was based on the concentration or amount of the herbicide present in the soil. It was observed that utilization was higher after four weeks of the experiment but after four weeks, the bacterial population started declining which may be because of the toxic nature of the herbicide or the high concentration of the herbicide. This shows the level of herbicide concentration which the microorganisms can withstand in the environment. The more herbicide applied to the soil in the process of removing unwanted weeds from farm the more the microbial population of the soil decreases.

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