Original Research Article

Glyphosate degradation by Two Plant Growth Promoting Bacteria (PGPB) isolated from rhizosphere of maize

Abstract

This study was aimed at evaluating the possible utilization of glyphosate tolerant plant growth promoting bacteria (*Pseudomonas aeruginosa* and *Bacillus cereus*) for bioremediation of glyphosate polluted soil. The soil samples were spiked with 3.1mg/ml, 7.2mg/ml and 14.4mg/ml of glyphosate and then inoculated with *Pseudomonas aeruginosa* and *Bacillus cereus*, level of glyphosate pollution before and after inoculation with the bacteria were determined using Gas Chromatography-Mass Spectroscopy (GC-MS) after extraction with acetonitrile. The bacteria showed significant ability to degrade glyphosate. *Pseudomonas aeruginosa, Bacillus cereus*, their mixed culture and control recorded percentage degradation of 76.11, 85.8, 75.8 and 49%, respectively at 3.1mg/ml of glyphosate while At the concentration of 7.2mg/ml, the percentage degradation by *P.aeruginosa, Bacillus cereus*, mixed culture of the isolates and control was 84.9, 72.7, 66.4% and 39.2%, respectively. The isolates also showed significant rate of degradation at the concentration of 14.4mg/ml.The GC-MS results showed a significant variation in the degradation products obtained when compared with control. This study revealed that substantial amount of glyphosate was degraded by *P.aeruginosa and Bacillus cereus*. Hence, they may have great potential in bioremediation of glyphosate polluted soil.

Keywords: Bioremediation, glyphosate, concentrations, PGPB

Introduction

Soil is one of the most important natural resource on which lives of all plants, animals and microorganisms directly or indirectly depend on. In soil, different microorganisms thrive on nutrients therein and through various interactions play a pivotal role in cycling of nutrients and pedogenesis (Ahemad and Khan, 2013). Alteration or disturbance in soil ecosystem by added pollutants leads to substantial changes in functional activities of these important soil microorganisms (Swain and Abhijita, 2013). The excessive use of glyphosate to control weed contributes in altering the natural environment due to the pollution of the environment by this persistent chemical. The mode of action of glyphosate involves the inhibition of the enzyme 5-enolpyruval shikimate-3- phosphate (EPSP) synthase in the shikimic acid pathway which is important in the biosynthesis of aromatic amino acids (Moneke *et al.,* 2010). This pathway exists in higher plant and microorganisms but not in animals (David and Topsy, 2001). By this mechanism, animals are believed not to be directly affected by glyphosate. However, the environmental consequences of the widespread use of the herbicide have been reported (Cox,

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2000). Several factors can affect the elimination of glyphosate in the environment, these factors includes size and activity of microbial population, soil structure, its adsorption ability, climate conditions, depth of motility in vertical soil profile etc (Shuskova *et al*., 2004). The environmental exposure to glyphosate is extensive due to the vast quantities used annually all over the world. Exposure could occur from direct application, accidental release or spray drift (David and Topsy, 2001). Glyphosate alters natural ecosystem by altering different components of soil microbial community (Inna *et al.,*2010), it inhibits the growth and decreases the activities of soil organisms (Carlise and Trevors, 1988).The main way of glyphosate degradation is by degradation by enzyme system of some microorganism(Strange-Hansen *et al*.2004).The utilization of plant growth promoting bacteria for biodegradation of glyphosate will not only reclaim the polluted soil but can as well enhance the fertility of the soil. These organisms enhance plant growth promotion through solubilization of insoluble nutrients in the soil and production of essential plant phytohormones. Plant associated bacteria, such as endophytic bacteria (non-pathogenic bacteria that occur naturally in plants) and rhizospheric bacteria (bacteria that live on and near the roots of plants), have been shown to contribute to biodegradation of toxic organic compounds in contaminated soil (Divya and Deepak, 2011). Less attention has recently been paid to bioremediation of contaminated soils with Plant growth promoting rhizobacteria(PGPR), Promotion of plant growth by bacteria is well documented (Babalola, 2010) and PGPR have been successfully used to reduce plant stress in contaminated soils. Some microbial communities have the ability to sequester some pollutants and therefore may also be useful in bio remediating contaminated soils (Hallberg and Johnson, 2005). Studies have shown that some PGPR can tolerate herbicides; therefore, this study is designed to assess the ability of PGPR to remediate herbicide polluted soil.

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Materials and Methods

Microorganisms and culture condition

Two glyphosate tolerant plant growth promoting bacteria were initially identified as *Pseudomonas aeruginosa strain* ZSL-2 and *Bacillus cereus strain* 20UPMNR .These isolates had been screened and had shown evidence of multiple plant growth promoting abilities. The isolates were maintained on nutrient agar slants at refrigerating temperature of 4^{o}C. Each seed culture was prepared accordingly by inoculating a loop of the stock culture into 50ml of nutrient broth after which the bacteria cells were harvested washed and re suspended in distilled water. To ensure

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equal cell population of each of the bacteria strain, their turbidity was adjusted to 0.5 McFarland standards.

Sample collection:

Soil samples were collected from research farm of the Institute of Agricultural Research and Training Moor plantation Ibadan.

Herbicide

The herbicide commonly known as Forceup manufactured by Zhejiang XinanChem Group Co.Ltd which contains 360g active glyphosate per litre was purchased from JubailiAgrotec Company, Ibadan.

Spiking of Soil with different Concentrations of glyphosate

The soil to be used was weighed, sieved and 5kg of soil were filled in perforated plastic pots, the herbicide (force up) was mixed with water and spiked on the soil samples until it reached the final concentration of 3.1, 7.2 and 14.4mg/ml. All the samples were thoroughly mixed with metal spatula. All treatments were laid in Complete Randomized Design (CRD) with three replicate.

Physicochemical analysis soil sample for screen house and field studies:

The physicochemical analysis such as moisture contents, pH, temperature, cation exchange bases, phosphorus,% total nitrogen, % total carbon, sodium, magnesium, potassium, sulphate, chloride etc were determined

Preparation of bacteria Inoculum

The isolates were inoculated in 50ml conical flask containing 25ml of prepared and sterilized luria broth and incubated at 30^{o}C in an orbital incubator shaker for 24 h. After incubation, the cultures were centrifuged at 4000rpm for 20mins.The cells were harvested and washed with normal cell. In other to ensure equal cell Size, the cells were diluted to 0.5 Mcfarlands Standard to give approximate cell density of 1.5 X10^{8}cfu/ml.

Collection of soil sample for Analysis of initial and residual glyphosate

Soil samples were collected from each pot immediately after application of herbicides and at the end of the experiment to determine the initial and residual herbicide. Initial and final soil samples were also collected in the field for analyses of initial and residual so as to validate the screen house studies..

Extraction of glyphosate from soil samples

The extraction of glyphosate from the soil samples were carried out by the method described by Frimpong*et al*. (2013), with slight modification from the Ghana Standard Authority (GSA) Herbicide Residues Laboratory Protocols. Ten grams (10 g) of the sub-soil samples were weighed and transferred into 250 ml separating flasks. A 10 ml of acetonitrile was added and the corked flasks sonicated (Grant XUB 18UK) for 5 min. An additional 10 ml of acetonitrile was added, and the separating flasks closed tightly. The content of the flasks were placed on a horizontal mechanical shaker (Ika-Werke HS 501 Digital), and was set to shake continuously for 30 min at 300 mot/min, and allowed to stand for 10 min to sufficiently separate the phases or layers. The supernatants (organic layers) were carefully transferred into 50 ml centrifuge tubes for centrifugation (Thermo/CR3i Multifunction) at 3000 rpm for 5 min. A 10 ml aliquot of the supernatants (organic phases/top layers) equivalent to 5.0 g soil weight were pipetted and dried/passed over 5 g anhydrous sodium sulphates through a filter paper into 50 ml round-bottom flasks. Then, 5 ml of acetonitrile was used to rinse the salt into the round-bottom flasks. The concentrates were then adjusted to about 2 ml using the rotary film evaporator (BuchiRatovapor R-210, USA) at 35 °C, and made ready for the analysis.

GC-MS Analysis*:*

GC-MS analysis was carried out on GCMS-QP2010 PLUS SHIMADZU. The column used was Perkin Elmer Elite - 5 capillary column measuring 30m × 0.25mm with a film thickness of 0.25mm composed of 95% Dimethyl polysiloxane. The carrier gas used was Helium at a flow rate of 0.5ml/min. 1µl sample injection volume was utilized. The inlet temperature was maintained as 250°C. The oven temperature was programmed initially at 80°C for 4 min, then an increase to 200°C. And then programmed to increase to 280°C at a rate of 20°C ending with a 5 min. Total run time was 35 min. The MS transfer line was maintained at a temperature of 200°c. The source temperature was maintained at 180°c. GCMS was analyzed using electron impact ionization at 70eV and data was evaluated using total ion count (TIC) for compound identification and quantification. The spectrums of the components were compared with the database of spectrum of known components stored in the GC-MS library.

Determination of percentage degredation

The percentage degradation of each treatment and control was estimated by considering the products containing the active ingredient present in the herbicide (glyphosate). The percentage degradation was calculated using the method of Adeyemi*et al*.,(2009).

Field Experiment:

The field experiment were carried out in a plot of land at the Institute of Agriculture Research and Training More Plantation Ibadan during 2017 farming seasons, with the experiment laid out in a complete randomized block design with three replicates and plot size of 2x3m. The experimental site is a plot of land with sandy loam soil, located at a latitude $7^{o}22$ $.701^{1}$N and longitude $3^{o}50.308^{1}$E. It is in the rainforest ecological zone of South west, Nigeria.

Land preparation and application of herbicides for field studies

The land were cleared and tilled prior to application of glyphosate(force up). Six hundred (600ml)of the water containing 3.1, 7.2 and 14.4mg/ml of glyphosate corresponding to half, field application rate and twice the field application rate were spiked on each of the 2x3m plot.

The method of Frimpong*et al*.,(2013) and GC MS were used for extraction and analysis of residual herbicides as earlier stated. Initial and final soil samples were also collected in the field for analyses of initial and residual glyphosate so as to validate the screen house studies. The residual herbicides were determined using GC MS after extraction. The percentage degradation was evaluated as earlier stated.

Data Analysis

Data were subjected to descriptive statistics, analysis of variance (ANOVA) and significant means were separated using Duncan Multiple Range Test (DMRT) at 5% level of probability.

RESULTS

Physicochemical parameters of the soil

The result of the physicochemical analysis of the soil sample from the field is presented on Table 32. The results showed range of parameters before and after planting as between: pH-5.91-6.19, Ca(cmo/kg)-1.73-2.54, Mg(cmo/kg)-0.76-1.49, K(cmo/kg)-0.14-0.22, % total carbon-0.67-1.01, % total nitrogen-0.04-0.07, %Organic matter-2.42, particle size-clay-13.4, sand-70.76 and silt-15.84(Table 1).

Biodegredation of glyphosate on the soil

The ability of the two isolates to degrade glyphosate at different concentration was tested. The percentage of glyphosate degraded in the soil by the isolates and their Chromatogram from GC-MS analysis at different concentrations are presented in fig.1 and 2.

Figure 1 shows the percentage degradation of 3.1mg/ml glyphosate by the isolate. The result showed that *P.aeruginosa, Bacillus cereus*, mixed culture of the isolates and control recorded percentage degradation of 76.11, 85.8, 75.8 and 49%, respectively. The GC-MS results showed a significant variation in the degradation products obtained when compared with control(Table2).

At the concentration of 7.2mg/ml, the percentage degradation by *P.aeruginosa, Bacillus cereus*, mixed culture of the isolates and control was 84.9, 72.7, 66.4% and 39.2%, respectively (Figure 2), whereas the percentage degradation by *P.aeruginosa, Bacillus cereus*, mixed culture of the isolates and control at the concentration of 14.4mg/ml was 47.15, 57.26, 55.7 and 27.4%, respectively. The result of degradation at 14.4mg/ml is presented in fig 3. The rate of degradation by the isolates decreased with increase in concentration of glyphosate except *P.aeruginosa* that showed higher rate of degradation at 7.2mg/ml when compared to 3.1mg/ml. There was also a a significant variation in the degradation products obtained when compared with control.The results of degradation products also showed transformation or total breakdown of some of the product found in the initial samples when compared with products recovered at the end of the experiment.

Discussion

Two glyphosate tolerant plant growth promoting rhizo bacteria namely, *P.aeruginosa* and *B.cereus*were used singly and combined to bio remediate glyphosate polluted soil. These isolate exhibited high ability to degrade glyphosate at different concentration. *B. cereus* recorded highest ability at the concentration of 3.1mg/ml and 14.4mg/ml while *P.aeruginosa* showed highest ability at the concentration of 7.2mg/ml, the least % degredation was recorded by the control in all the concentration. The rate of degredation was lower when the isolates are mixed than when used singly. This might be as a result of antagonistic interaction between the two isolates which may have interfered with their metabolic activities. The ability of the isolates to

degrade glyphosate may be connected with its ability to utilize glyphosate as C and P source since the degredation of glyphosate involves the lysis of C-P bond. The two pathways for glyphosate degredation involves cleaveage AMPA and glyoxylate by the presence of glyphosate oxidoreductase where as in the other pathway, degredation is catalyzed by C-P lyase with the formation of sarcorsine which eventually forms formaldehyde and glycine (Sviridov*et al*.,2011). The findings of this research is line with Haoyu*et al*.,(2015) who reported the degradation of glyphosate by Pseudomonas sp., Jacob *et al*.,(1988) isolated a Pseudomonas strain which completely metabolized 3.21g/l glyphosate with a degrading efficiency of about 2gGp/g dry biomass. Two bacteria strains of bacteria were reported to be efficient degraders of glyphosate, these isolates are Ochrobacteriumanthropic(Sviridov*et al*, 2011) and Bacillus cereus CB4(Fan *et al*.2012). These two isolates were reported to degrade glyphosate through the two pathways mentioned earlier.

Comment [R6]: ?

Luft*et al*.,(2017) reported glyphosate degradation by two plant growth promoting bacteria *Enterobacter* sp and *Pseudomonas fluoresces*. The isolates used in this study have two functions as plant growth promoting bacteria and degradation of glyphosate herbicide. These characteristics are quite beneficial to humans where bacteria can help to reduce levels of glyphosate that has high persistence and poisonous to plant and beside has plant growth promoting properties that can increase crop yield (Luft*et al*., 2017). Glyphosate degradation also depends on the adaptation of bacteria to herbicides, phosphate status in bacteria cell and bacteria culture growth phase(Kryuchkova *et al*.,2014). Travaglia*et al*,(2015) reported that Pseudomonas and Azospirilum are capable of detoxifying glyphosate because the undergo longer stationary phase and delayed phase of death. The report of our findings is also in line with the findings of Inna *et al*.,(2010) who reported that high degradation of glyphosate using degraders belonging mostly to the genera Pseudomonas, Arthrobacter and Alcaligenes isolated from different source including soil. The report of the percentage degradation by the mixed culture of the isolates agreed with findings of Romeo and Hendawi (2014) who reported higher efficiency in herbicide degradation by *A. lipoferum* when used singly(48.3%) than when combined with *B.polymyxa* (46.8%). Yu *et al*.,(2015) reported 17.65-66.97% degradation of glyphosate in sterile soil and 19.01-71.57% in unsterilized soil using *Bacillus subtilis.* The effectiveness of *Bacillus* sp., Citrobacter and *P. flourescens* to degrade glyphosate up to 50mg/l concentration were also reported (Abubacker*et al*.,2016). The initial and final GC-MS analysis of the polluted soil

showed transformation or total breakdown of the components of the herbicides. Most of the products of the initial samples were not found at the end of the experiment where as some new compound were seen at the end of the experiment were not in the initial sample. This is also in line with the findings of Abubacker*et al*.,(2016) who reported the transformation of the components of glyphosate during its degradation at the end of his experiment. The transformation may be as a result of microbial action or plant enzymes. The simultaneous cleanup of herbicides using chemical and thermal methods are both technically difficult and expensive, these methods also destroys the biotic components of the soil. The utilization of plant growth promoting bacteria will enhance the biodegradation as well restore soil and biotic components (Abdel megeed, 2013). These PGPB can be useful in the process of soil clean up after glyphosate application to prevent accumulation of glyphosate in the soil and the reduction of its toxicity. The results of this work has revealed the ability of the two isolates to effectively degrade glyphosate without accumulation of amino methyl phosphonic acid (AMPA) as a metabolic product. Hence this isolates can be employed in bioremediation of glyphosate polluted soil.

Conflict of interest: NA

Ethical standard: NA

References

Abubakar, M.N.,Visvanathan, M and Srinivason.(2016).Biodegredation of glyphosate herrbicides by bacterial isolates from Banana(*Musa*spp) plantation soil. *International quarterly Journal of Biology and Life Science*, **4**:243-250.

Abdel-megged, A., Sadik,M.W.,Al-sharani, H.O and Ali,H.M.(2013). Phyto microbial degradation of glyphosate in Riyadh area. *International Journal of Microbiology Research*.**5**:458-466.

Ahemad M and Khan M.S. (2013). Pesticides as antagonists of rhizobia and the legume-Rhizobium symbiosis: a paradigmatic and mechanistic outlook. *Biochemistry and Molecular Biology*,**1**:63–75.

Babalola, O.O.(2010). Beneficial bacterial of agricultural importance.*Bio technology letters*. **32**:1559-1570.

Carlisle S.M. and Trvors J.T.(1988).Glyphosate in the Environment. Water, Air and Soil Pollution,**39**:409-420.

Cox, C.(2000). Glyphosate fact sheet.*Journal of Pesticide reform*,**108**:45-51.

David, B. and Topsy, J. (2001). Health and environmental impacts of glyphosate.Friends of the earth pesticide action network UK.

Divya, B and Deepak, K.M.(2011). Plant–Microbe Interaction with Enhanced Bioremediation.*Research Journal of BioTechnology*.**6**:72-79.

Fan, J. Y., Yang, G. X., Zhao, H. Y., Shi, G. Y., Geng, Y. C. (2012).Isolation, identification and characterization of a glyphosate-degradingbacterium, *Bacillus cereus* CB4, from soil. *Journal of General and AppliedMicrobiol*ogy, **58**: 263–271.

Frimpong KS, Gbeddy G, Doyi I, Arye-Quaye F, Kokroko W, Asamoah CO. (2013). Efficient method development for atrazine determination in soil samples. An Indian Journal of Environmental Sciences ;8:264–267

Haoyu, Z., Ke, T., Jianyi, Z., Shengnan, L., Han, G and Xdiaogang, Z. (2015).Bioremediation potential of glyphosate degrading Pseudomonas spp. Strains isolated from contaminated soil. *Journal of General Microbiology*.**61**:165-170.

Inna, T.E., Nina I.K.,Tatyana S, Mikhail, Z. and Gennady, A.Z. and Alexey, A.L. (2010). Bioremediation of glyphosate contaminated soil. *Applied microbiology and Biotechnology*,**88**: 585-594.

Jacob, G.S., Garbow, J.R., Hallas, L.E., Kimack, N.M., Kishore, G.M. and Schaefer, J. (1988).Metabolism of glyphosate in Pseudomonas sp. strain LBr. *Applied Environmental Microbiology*.**54**: 2953 – 2958.

Lufti, T.A.,Lugman, A. and Tutung, H. (2017).Glyphosate biodegradation by plant growth promoting bacteria and their effects to paddy germination in glyphosate contaminated soil. *Journal of degraded and mining land management,* 5:995-1000.

Moneke, A.N., Okpala, G.N. and Anyanwu, C.U. (2010).Biodegradation of glyphosate herbicidein vitro using bacterial isolates from four rice fields.*African Journal of Biotechnology*, **9**:4067 – 4074.

Romeh, A.A. and Hendawi, M.Y.(2014). Bioremediation of certain organophosphorus pesticides by two biofertilizers, Paenibacillus(Bacillus), Polymyxa(Prazmowski) and Azospirilliumlipoferum(Beijerinck). Journal of Agric science technology, **16**:265-276

Shushkova, T., Ermakova, I. and Leontievsky, A.A. (2010).Glyphosate bioavailability in soil. Biodegredation, **21**:403-410.

Sviridov, A. V., Shushkova, T. V., Zelenkova, N. F., Vinokurova, N. G.,Morgunov, I. G. (2011). Distribution of glyphosate andmethylphosphonate catabolism systems in soil bacteria*Ochrobactrum anthropic* and *Achromobacter*sp. *Applied Microbiology and Biotechnol*ogy, **93**:787–796.

Swain, H and Abhijita, S. (2013). Nitrogen fixation and its improvement through genetic engineering.*Journal of Global Biosciences*, **2:**98–112.

Travaglia, C., Masciarelli, O., Fortuna, J., Marchetti, G., Cardozo, P., Lucero, M., Zorza, E., Luna, V. and Reinoso, H. (2015). Towards sustainable maize production: glyphosate detoxification by Azospirillum sp. and Pseudomonas sp. Crop Protection,**77**: 102-109.

Yu, X.M., Yu, T., Yin, G.H., Dong,Q.L., An, M., Wang, H.R. and Ai, C.X. (2015).Glyphosate biodegradation and potential soil bioremediation by *Bacillus subtilis* strain Bs-15. *Genetic and molecular resources*,**14**:14713-30.

Table 1: Physicochemical parameters of the soil before and after planting

Parameters	Value
PH	5.93
Ca (cmo/kg)	1.73
Mg(cmo/kg)	0.96
K(cmo/kg)	0.97
Na(cmo/kg)	0.28
H^+	0.11
Electrical conductivity (µs)	62.37
P(ppm)	13.24
%Total carbon	1.41
% Total nitrogen	0.14
% Organic matter	2.42
Cupper(PPM)	1.89
Fe(PPM)	129.56

Mn(PPM)	75.03
Sulphur(PPM)	10.32
Boron(PPM)	0.10
Zinc(PPM)	3.03
CEE(Cmo/kg)	4.06
% Base saturation	97.20
Particle size-Sand	70.76
Silt	15.84
Clay	13.4

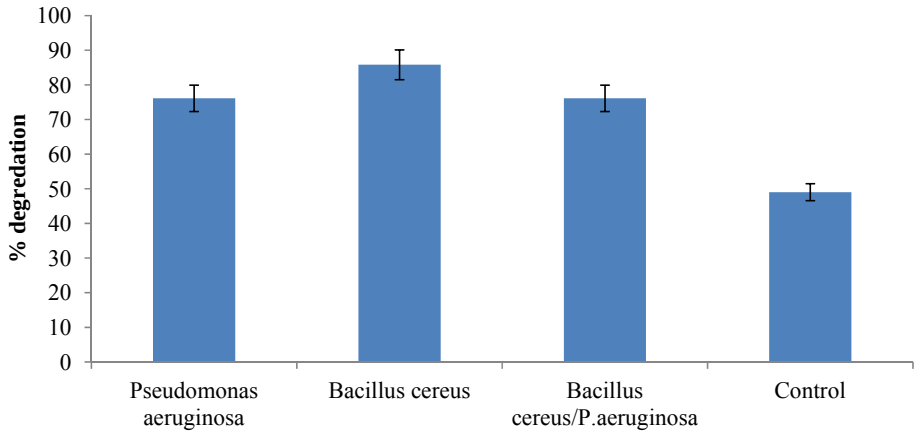

Fig 1:Percentagedegredation of glyphosate by the isolates at concentration of 3.1mg/g

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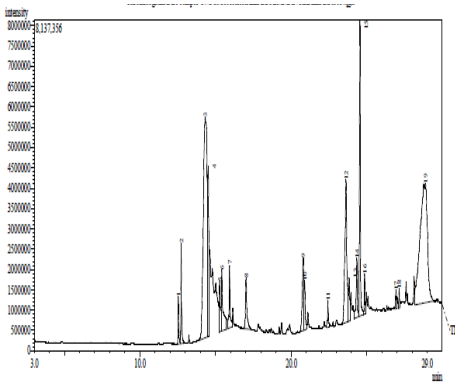
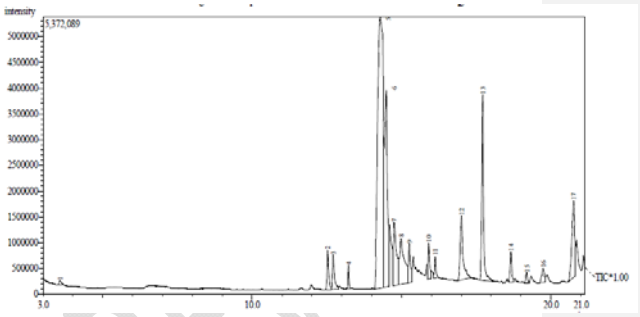
336 A-Initial sample B-Innoculated with *P.aeruginosa*





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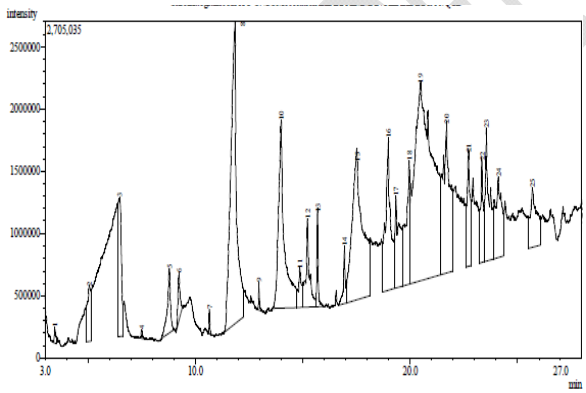
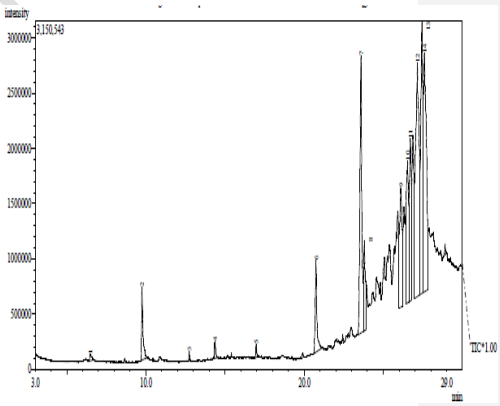
338 C-Innoculated with *B.cereus* D-Innoculated with mixed

339 culture





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E-Control

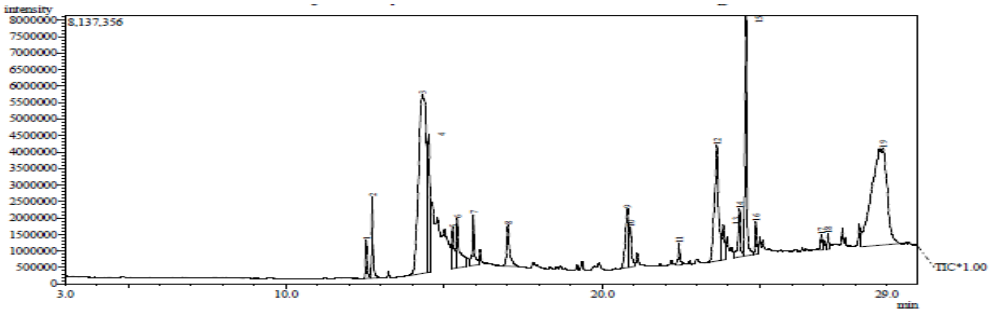


Fig 2: Chromatogram of GCMS analysis of soil spiked with 3.1mg/ml

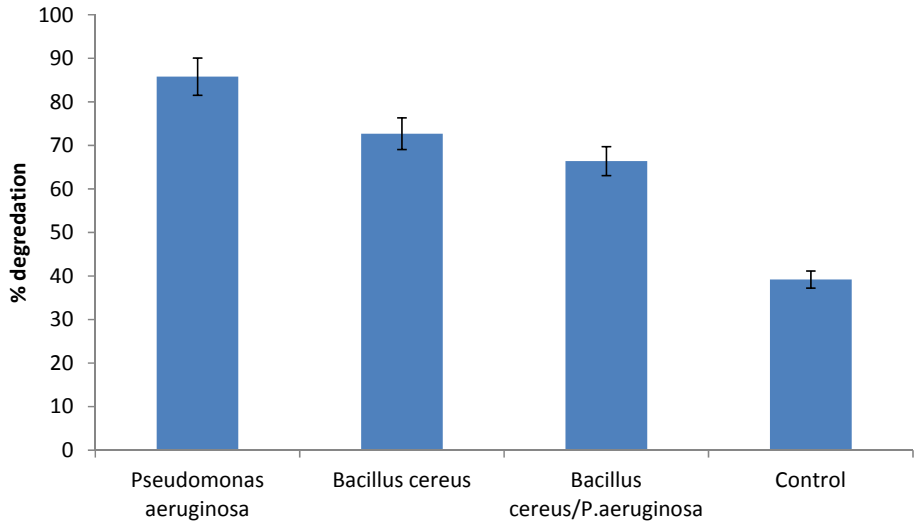
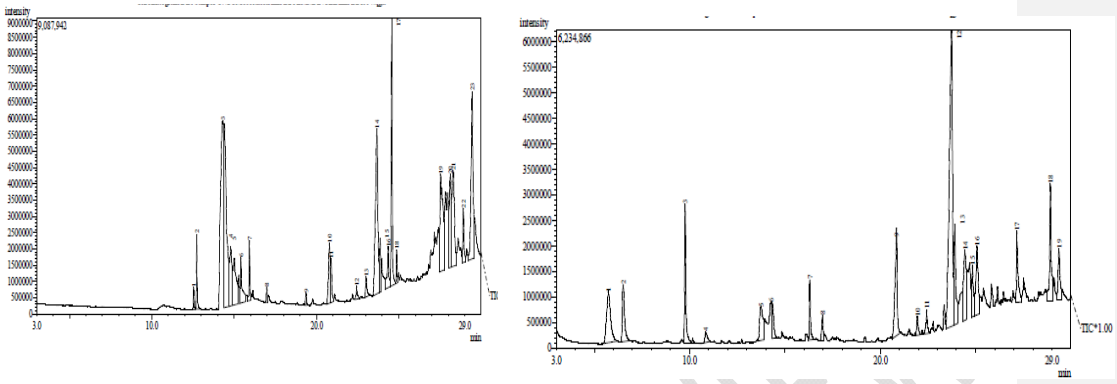


Fig 3:Percentagedegredation of glyphosate by the isolates at concentration of 7.2mg/g

A-Initial sample B-Innoculated with *P.aeruginosa*



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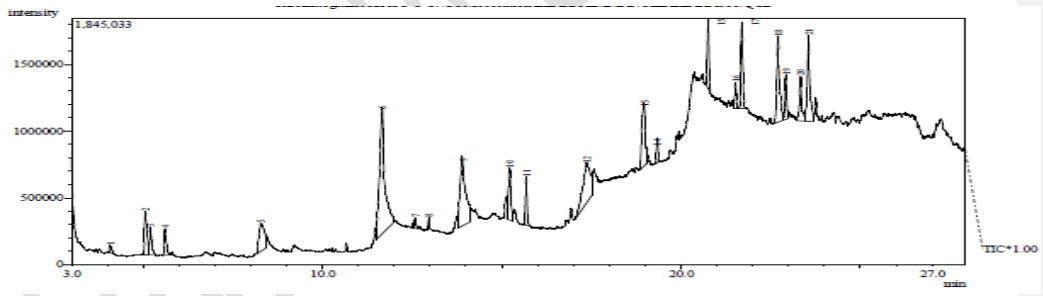
354 C-Innoculated with *B.cereus*



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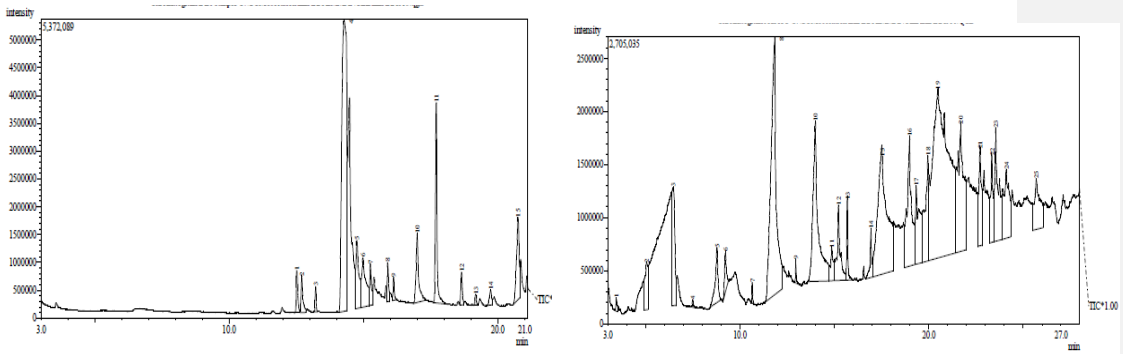


D-Innoculated with mixed culture E-Control

Fig 4: Chromatogram of GCMS analysis of soil spiked with 7.2mg/ml

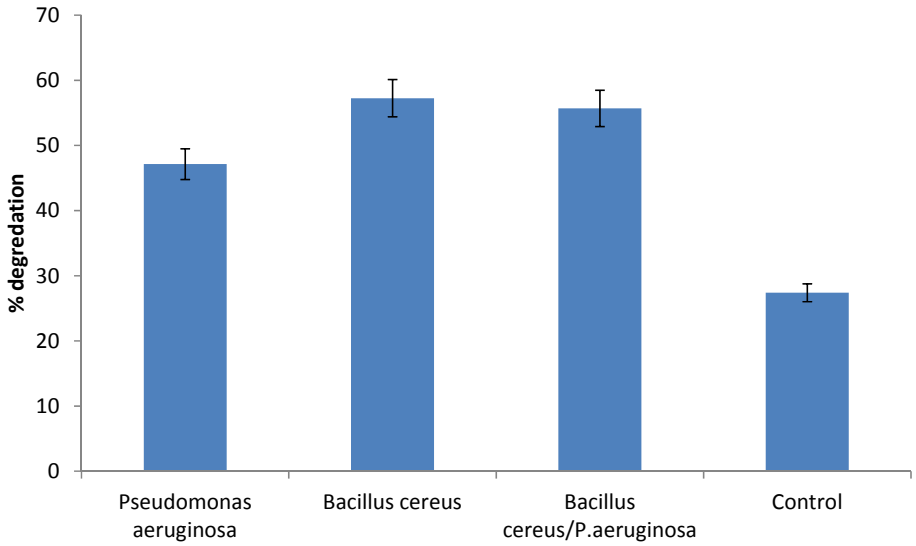
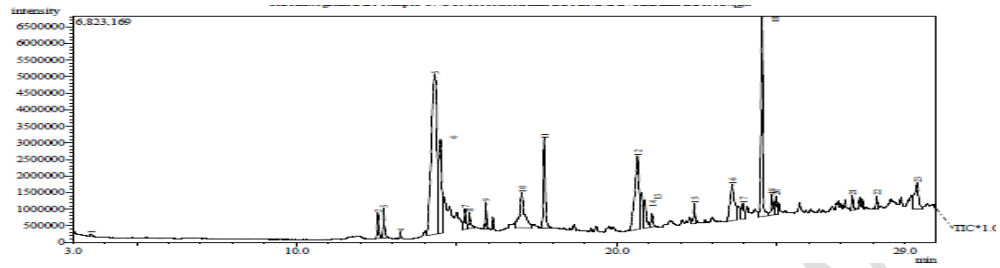


Fig 5:Percentagedegredation of glyphosate by the isolates at concentration of 14.4mg/g



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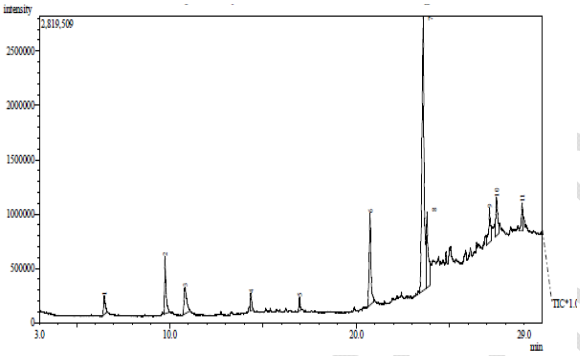

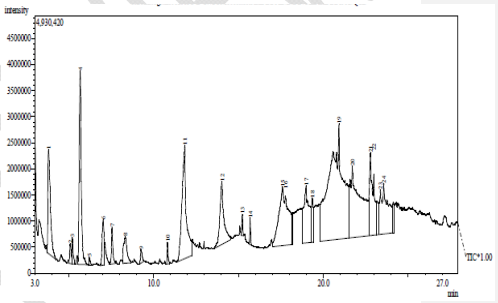

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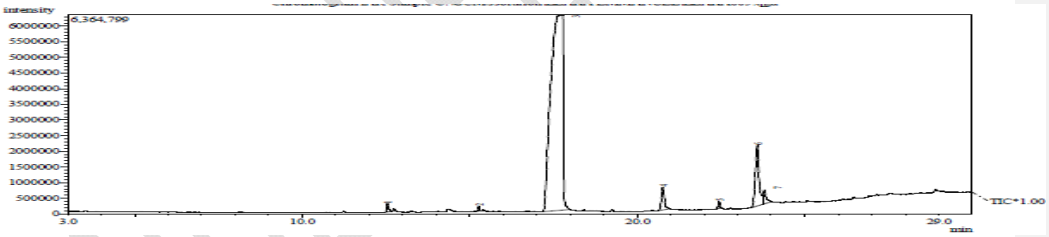

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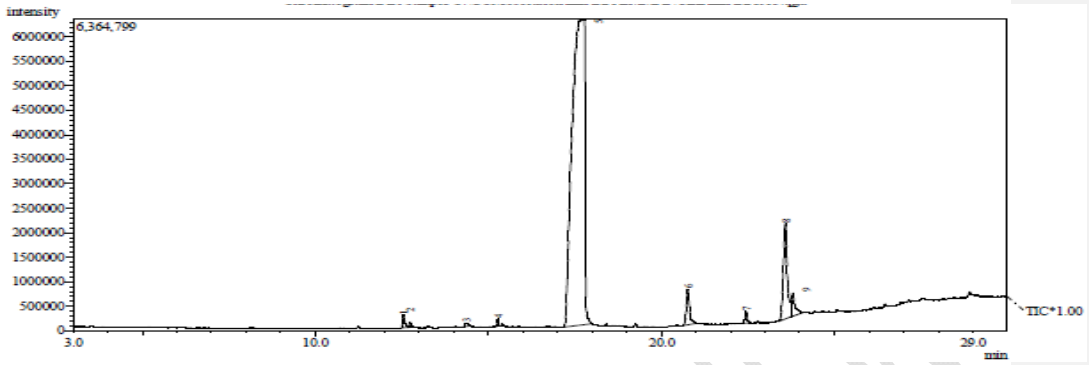

Fig 6: Chromatogram of GCMS analysis of soil spiked with 14.4mg/ml of glyphosate

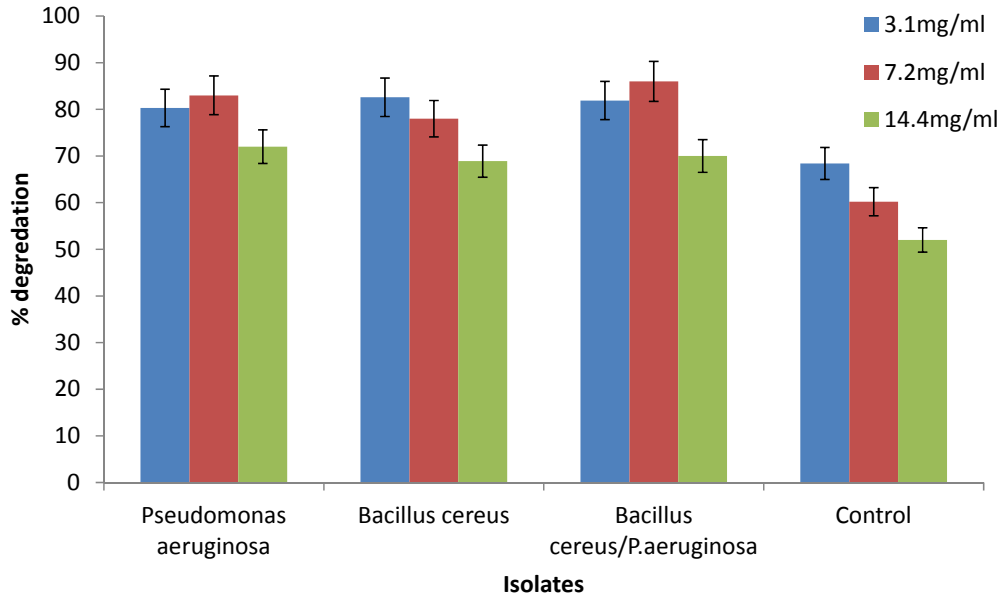

Fig.7: Percentage degredation of glyphosate by the isolates at different concentration of glyphosate in the field

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378 **Table 2: Initial and degredative product of glyphosate in the soil**

Before degredation	Mol. weight	Formular	After degredation	Mol. weight	Formular
pyridine	93	C_6H_7N	2-Butylene Himidazole	122	$C_7H_{10}N_2$
6methyl -picolinic acid	137	$C_7H_2NO_2$	4-pentyl-2-tuylamine	123	$C_8H_{13}N$
N N-isophthaloylbis	404	$C_{22}HN_{206}$	2-Propanamine N-methyl ethylidene	99	$C_6H_{13}N$
Dodecane(1-chlorodecylchloride)	176	$C_{10}H_{21}Cl$	Pyrolidine	157	$C_8H_{15}NO_2$
1-chlorononyl chloride	176	$C_9H_{19}Cl$	2-Propanomine	99	$C_6H_{13}N$
Dodecanol	186	$C_{12}H_{26}O$	3-Azonia 5-hexene-1-ol	173	$C_8H_{17}N_{202}$
Dodecene	168	$C_{12}H_{24}$	4-piperidinone	155	$C_9H_{17}NO$
Tridecene	154	$C_{13}H_{26}$	2-pentanamine	129	C_8H1_9N
Hexanoic acid	158	$C_9H_{18}0_2$	2,2,5,5 tetrmethyl 4 ethyl imidazole	172	$C_9H_2ON_2O$
Heptanoic acid	144	$C_8H_{16}O_2$	Pyrolidinone	99	C_5H_9NO
Bipyrine	156	$C_{10}H_8N_2$	Pyridazine	204	$C_9H_{12}N_6$
NN-Isopropyl N-4-butyl guanidine	240	C13H28N4	Silane	144	$C_{22}H_{45}Cl_3SI$
			2H Pyrol-2-one	97	C_5H_7NO

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