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Determination of the Efficiency of the Mixture of Isolated Bacterial Species in Bioremediation of Pesticides

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ABSTRACT

In the present investigation results showed a different mixture of bacteria with pesticides at 25 ppm *Sa.bongori* and *S.aureus* removing pesticides 64.8% for Atrazine, 74.4% for Diazinon and 75.7% for Pirimicarb , As for *E.coli* and *S.aureus* It showed on Atrazine 66.8%, Diazinon 76.3% and Pirimicarb 77.7%. Also *E.coli* and *Sa.bongori* resulted in 60.5% removal of atrazine, 72.7% for Diazinon and 49.7% for pirimicarb. When the three species of bacteria were mixed together, their combined effect was 83.4% for Pirimicarb bioremediation 82.0% for Diazinon and 69.1% for Atrazine.when comparing the growth rate of bacterial cells, The mixture was *E.coli* and *Sa.bongori* 31.52×10^4 to increase in Diazinon and Pirimicarb 32.01×10^4, Pirimicarb and atrazine 32.23×10^4, Diazinon and atrazine 31.98×10^4 mixture of three pesticides 40.54×10^4. Also mixture *S.aureus* and *Sa.bongori* Before treatment 22.86 × to become after in Diazinon and Pirimicarb 37.32 × , Pirimicarb and atrazine 44.13 ×, Diazinon and atrazine 35.68×10^4 mixture of three pesticides 43.65×10^4, Finally, the mixture recorded all bacteria growth from 34.12×10^4 To grow in Diazinon and Pirimicarb 51.18×10^4, Pirimicarb and atrazine 40.54×10^4, Diazinon and atrazine 45.38×10^4 mixture of three pesticides 52.36×10^4. This method has demonstrated that mixing bacterial species on biological treatment of pesticides increases the speed of the process as each bacterium completes the work of the other, which contributes to the removal of pesticides, and can be used safely in the process of pesticide removal, however more research is needed on safety, mechanisms and mobility.

Introduction

Pesticides are chemical compounds that are used to combat pests, including insects, rodents, fungi and unwanted plants (weeds). Pesticides are used in public health to fight vectors of disease, such as mosquitoes, and in agriculture, to combat pests that damage crops. By their nature, pesticides are potentially toxic to other organisms, including humans, and need to be used safely and disposed-off properly (WHO, 2019) pesticides are applied to agricultural crops annually for pest control worldwide. It is estimated that less than 1% of the total applied pesticides generally gets to the target pests and most of the pesticides remain un-used and enter into the ecosystem.

The ultimate sink for excessive pesticides is soil and water. (Kuhad, *et al.*, 2013).There is a vital need to remediate and clean heavily polluted soil with pesticides and pesticides residues.

Among various soil remediation technologies available today for decontamination and detoxication of pesticide-contaminated soils, bioremediation seems to be one of the most environmentally- safe and cost- effective methods. Bioremediation refers to the use of microorganisms (Bacteria, fungi) or green plant to degrade contaminants that pose environmental and human risks.

The versatility of microbes to degrade a vast array of pollutants makes bioremediation processes typically involve the actions of many different microbes acting in parallel or sequence to complete the degradation process. Bioremediation is a technology that can be applied in different conditions.

Though it can be inexpensive and *in situ* approaches can reduce disruptive engineering practices, bioremediation is still not a common practice (microbewiki, 2018). Bacteria are widely diverse organisms, and thus make excellent players in biodegradation and bioremediation.

There are few universal toxins to bacteria, so there is likely an organism able to breakdown any given substrate, when provided with the right conditions (Anaerobic vs. aerobic environment, sufficient electron donors or acceptors, etc.) (microbewiki, 2018).

Hence, the present study was carried out to Isolation and characterization of bacterial species that have ability to bioremediation of pesticides.

Determine the efficiency of mixture of isolated bacterial species on bioremediation of pesticides diazinon, pirimicarb and atrazine. Evaluation of the level of pesticide removal by mixture of bacterial species and Comparison of growth rate of bacterial cells in pesticides.

Materials and Methods

Sample collection

The soil samples were collected from farm to the western side of the University of Gezira at 14.3858° N, 33.5294° E in Wad Medani city, Sudan.

Design and Statistical Analysis

The experimental layout was a randomized complete block (RCB) design in split plot system, with three replicates. Data was subjected to ANOVA using the Statistical Analysis System (CoStat's) Statistical Procedures and treatment means were compared using the revised L.S.D. test at a 0.05 level according to (Robert George and Douglas Steel, 1997).

Pesticides Used in This Study

Three concentrations were prepared from the standard pesticide solution 100 ppm, *i. e.* 10 ppm, 25 ppm and 50 ppm**.**

Table.1

Pesticide	Group	Scientific name	Chemical formula
Diazinon	OP	*O,O*-Diethyl *O*-[4-	$^{C}12^{H}21^{N}2^{O}3^{P}$
		methyl-6-(propan-2-yl)	S
		pyrimidin-2-yl]	
		phosphorothioate	
Pirimicar	carbamate	2-dimethylamino-5,6-	$^{C}11^{H}18^{N}4^{O}2$
b		dimethylpyrimidin-4-yl)	
		N,N-dimethylcarbamate	
Atrazine	triazine	6-chloro-N2-ethyl-N4-	$C_8H_{14}ClN_5$
		(propan-2-yl)-1,3,5-	
		triazine-2,4-diamine	

Isolation and identification of bacterial Isolates

Serial folds dilution technique was used for the isolation of pesticide degrading bacteria in nutrient agar. Well grown bacterial colonies were picked and further purified. The purified isolates were identified according to criteria described by Barrow and Feltham (2003). This included staining reaction, organism morphology, growth conditions, colony characteristics on different media, and biochemical characteristics.

Counting Bacterial Cells

1. Total viable cells.
2. Total nonviable cells.
3. Percentage of viable cells:

% of viable cells = $\frac{\text{viable cells}}{\text{total of cell}} \times 100$

4. Average of cell / square: = $\frac{\text{viable cell}}{\text{squaer}}$

5. Dilution factor: = $\frac{\text{final volume}}{\text{volume of cell}}$

6. Concentration (viable cell / ml) = $\frac{\text{average of cell}}{\text{square}} \times$ dilution factor $\times 10^4$.

Bioremediation process of pesticides by isolated bacteria

The tubes are equipped with autoclave for 40 min at 120 °C and Activation of bacteria. The vaccine was prepared by adding 1-3 colonies of bacteria in normal saline 8. Five g of NaCl. Then Ten ml of Broth Culture Liquid media was placed in each tube. 1 ml of pesticides at the required concentrations (10 ppm - 25 ppm - 50 ppm) was added.1 ml of bacteria solution to the tubes was added. After that The incubation process was done by placing the tubes at 37 °C in a shaking water bath The results were taken after 24 hr. by taking 5 ml of the treated solution after excluding the leachate and taking the top extracted by centrifuge. Finally 5 ml acetonitrile (CAN) was added to stop the activity of the bacteria in the extract.

Processing of Samples for Separation and Extraction Processes

After extracting 5 ml of the sample solution, QuEChERS extraction materials were added to the sample, consisting of 4 mg MgSO4 and 1 NaAC. The samples were then placed in a centrifuge for 5 minutes at 4000 rpm and the supernatant was withdrawn from the samples. Then the samples were concentrated using 0.5

mL nitrogen. The calibration curve concentrations were prepared to determine the accuracy of the experiment into the GC/ MS and analyze.

Calculation of Pesticides decomposition rate

$$\frac{C1-C2}{C1} \times 100$$

C1 = Concentrate Solution before Treatment
C2 = Concentrate Solution after Treatment

Isolation and characterization of bacterial species that have ability to bioremediation of pesticides

The bacteria were identified *Staphylococcus aureus, Salmonella bongori* and *Escherichia coli* from soil to use for the bioremediation of pesticides. The results of analysis of the biochemical properties of bacterial species isolated from different samples are shown in the Table (1).

Determine the efficiency of the mixture of isolated bacterial species on bioremediation of pesticides diazinon, pirimicarb and atrazine

A mixture of the tested pesticides was prepared at a 25ppm and was treated with a mixture of *E. coli* and *S. aureus*. After 24 hr, the results showed no significant differences. However, the pirimicarb resulted 77.7% degradation, diazinon 76.2%, while atrazine was 66.8%. As depicted (Fig 1) The mixture of *S. aureus* + *Sa. bongori* resulted in 75.7% for pirimicarb, 74.4% for diazinon and 64.8% for atrazine (Fig 2). For the treatment of a mixture of *E. coli* + *Sa. bongori*, the pirimicarb showed 71.2% decomposition rate, diazinon removal rate was 70.4% and, finally, atrazine degradation rate was 59.6% (Fig 3). It was noted that when pesticides treated with a mixture consisting of the three species, *the highest level of degradation was obtained* on pirimicarb, which reached 83.4%, and 82% for diazinon, whereas atrazine reached up to 69% (Fig 4). Comparisons among the mean values of the efficiency of the bacterial mixture in the bioremediation, showed significant differences.

The highest significant value of this trait was recorded in all tested bacterial species for degradation rate of 78.16%, while the lowest significant value was noticed in the *E. coli* and *Sa. bongori* (60.98%). The *E. coli* and *S. aureus* degradation rate of 73.60%, while the *S. aureus* and *Sa. bongori* resulted in 71.64%. However; there were no significant differences between the efficiency of the three species Table (2). In Table (3) the highest significant value of interaction between the mixture of these bacteria and the tested pesticides in the bioremediation of these pesticides was recorded by these bacteria with pirimicarb and with diazinon, while the lowest value was found for *E. coli* and *Sa. bongori* with pirimicarb.

Evaluation of the level of pesticide removal by mixture bacterial species

According to the data given the diazinon and pirimicarb indicated significantly the highest mean values, while atrazine was the lowest mean value when treating pesticides with the mixture of the three bacterial species Table (4).

Comparison of growth rate of bacterial cells in pesticides

E. coli +*S. aureus*: The growth rate was 32.06 $\times 10^4$ before treatment. But, on the mixture diazinon + pirimicarb the rate was 48.09 $\times 10^4$, pirimicarb +atrazine increased to 54.64 $\times 10^4$ and for diazinon + atrazine it resulted in 43.35×10^4. The mixture of the 3 pesticides effected 45.11×10^4(Fig 5).

E. coli+Sa. bongori: Under this condition 31.52×10^4 was reported. This was increased in diazinon + pirimicarb to 32.01×10^4, pirimicarb + atrazine 32.23×10^4 and diazinon + atrazine to 31.98×10^4. However, the mixture of three pesticides resulted in even higher rate 40.54× 10^4 (Fig 6).

S. aureus + Sa. bongori: Before treatment rate was 22.86×10^4, to become in diazinon + pirimicarb 37.32×10^4, pirimicarb + atrazine 44.13 × 10^4, and diazinon + atrazine 35.68×10^4. The mixture of three pesticides rate was 43.65 × 10^4 (Fig 7) the mixture the three species. The growth rate under this condition was 34.12 ×10^4. It became in diazinon + pirimicarb 51.18 × 10^4, pirimicarb + atrazine 40.54 × 10^4, and diazinon + atrazine 45.38 × 10^4. However, the mixture of three pesticides gave the highest rate 52.36 × 10^4(Fig 8)

Results and Discussion

Through the results obtained from the process of mixing bacterial species and the treatment of a mixture of pesticides to simulate nature, found that the mixture containing the all bacteria type shows the highest efficiency by 78.16%. This indicates that there is an integrative role between different bacterial species. This is followed by a mixture containing bacteria E. coli and S. aureus 73.60%, and mixture of Sa.bongori and *S.aureus* 71.64%. Although there were no significant differences between them, followed by the mixture E. coli and *Sa.bongori* which is considered to be less efficient 60.98% combination.

It is noted in these results, the mixtures containing bacteria S.aureus are the highest efficacious ones, this indicates its high-capacity in pesticide degradation.

Evaluation of pesticide removal efficiency

Focusing on the results obtained from the treatment of pesticides with bacteria mixtures. It was found that the Atrazine pesticide reported the lowest decomposition rate of 65.03% compared to Diazinon 76.33% and Pirimicarb71.65%.

Based on the results obtained, it can be said that the Atrazine pesticide has a relatively simple decomposition characteristic, these results are consistent with kookana *et al.,* (1994) they concluded on others pesticides such as Atrazine and Simazine are biodegradable at slow rates and may by leached from soil to ground water.

Table.1 Biochemical test of *Escherichia Coli, Salmonella bongori* and *Staphylococcus aureus*

Tests	*Sa.bongori*	*E.coli*	*S.aureus*
Indole	-	+	-
Methyl Red (MR)	+	+	+
Urease test	-	-	+
Catalase	+	+	+
Motility	+	+	-
Citrate	+	-	-
Gram test	-	-	+

Table.2 Determination of the efficiency of the mixture of isolated bacterial species in bioremediation of pesticides

Mixture of bacteria	Result (%)
All species	78.16 a
E. coli + *S. aureus*	73.60 ab
S. aureus + *Sa. bongori*	71.64 ab
E. coli + *Sa. bongori*	60.98 b

Values having the same letter (s) are not significantly different from one another, using revised L.S.D. test at 0.05 level of probability

Table.3 Effect of interaction between the mixture of the tested bacterial species and three tested pesticides in the bioremediation of these pesticides

Mixtures of bacteria	Pesticides	Result (%)
All bacteria	pirimicarb	83.40 a
All bacteria	diazinon	82.00 a
E.coli + *S.aureus*	pirimicarb	77.70 a
E.coli + *S.aureus*	diazinon	76.26 a
S.aureus + *Sa.bongori*	pirimicarb	75.733 a
S.aureus + *Sa.bongori*	diazinon	74.40 a
E.coli + *Sa.bongori*	diazinon	72.66 a-b
All bacteria	atrazine	69.06 a-b
E.coli + *S.aureus*	atrazine	66.80 a-b
S.aureus + *Sa.bongori*	atrazine	64.80 a-b
E.coli + *Sa.bongori*	atrazine	60.53 a-b
E.coli + *Sa.bongori*	pirimicarb	49.73 b

Values having the same letter (s) are not significantly different from one another, using revised L.S.D. test at 0.05 level of probability

Table.4 The efficiency of the decomposition of pesticides with a mixture of the test bacterial species

Pesticides	Result (%)
Diazinon	76.33 a
Pirimicarb	71.65 ab
Atrazine	65.3 b

Values having the same letter (s) are not significantly different from one another, using revised L.S.D. test at 0.05 level of probability

Fig.1 Effect of *E. coli* + *S. aureus* on the mixture of the pesticides

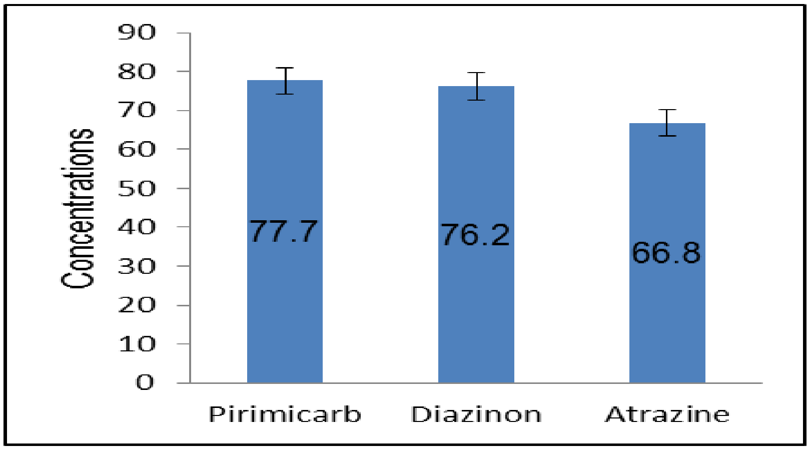


Fig.2 Effect of *S. aureus* + *Sa. bongori* on the degradation of the mixture of the pesticides

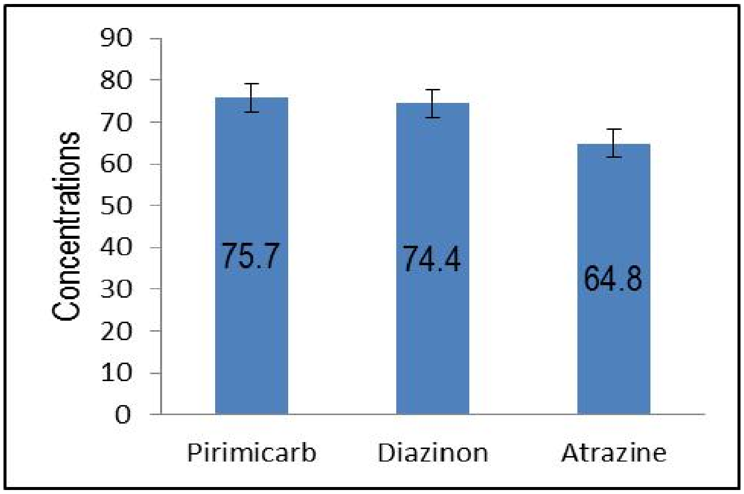


Fig.3 Effect of *E. coli* + *Sa. bongori* on the degradation of the three pesticides

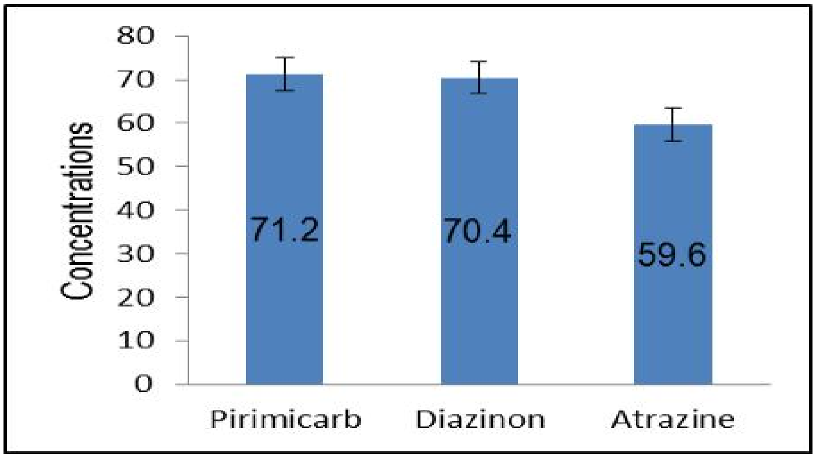


Fig.4 Effect of the three bacterial species on the degradation of the three pesticides

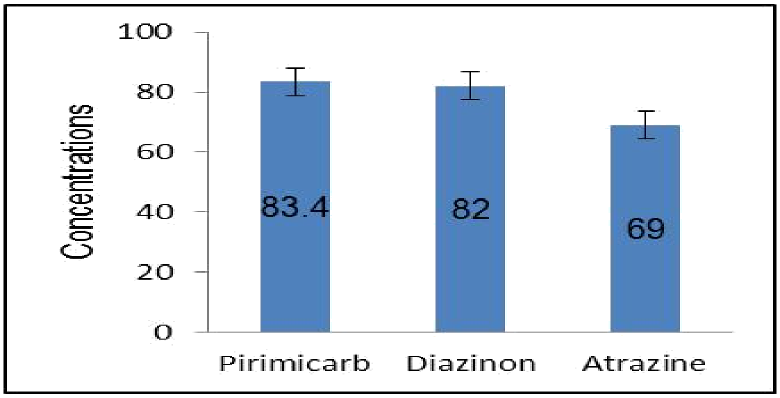


Fig.5 *E. coli + S. aureus* growth rate before treatment (blue) and after 24 hr (red)

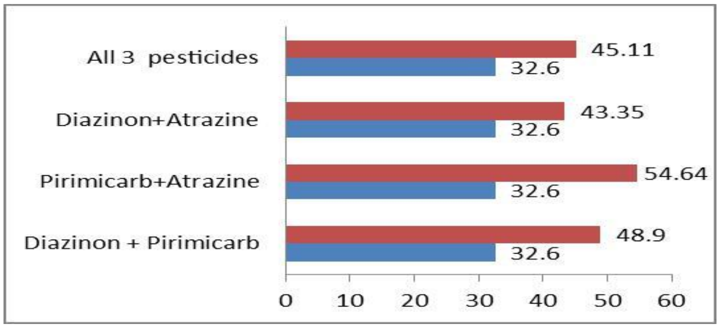


Fig.6 *E. coli + Sa. bongori* growth rate before treatment (blue) and after 24hr (red)

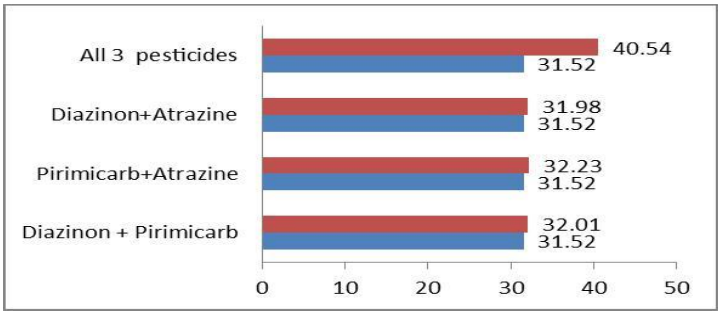


Fig.7 *S. aureus + Sa. bongori* growth rate before treatment (blue) and after 24hr (red)

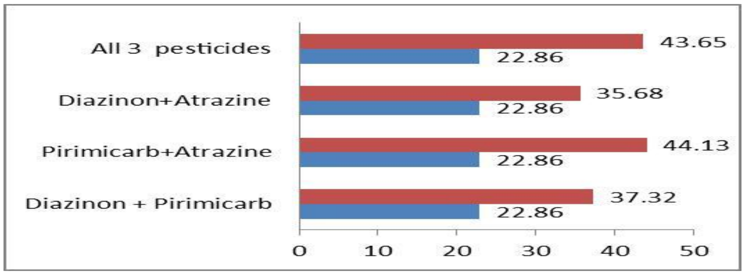
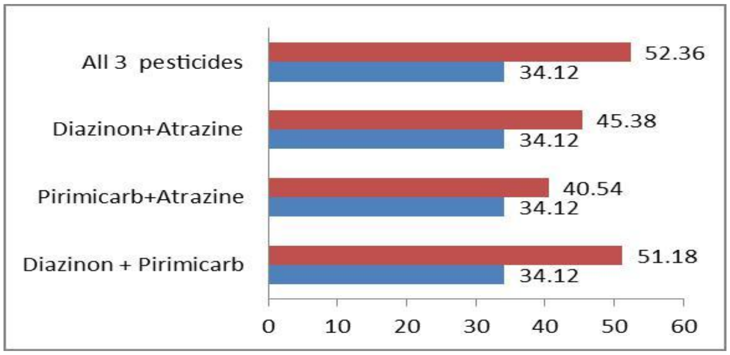


Fig.8 The three bacterial species growth rate before treatment (blue) and 24 hr (red)



Conversely, in the present study found that pesticide diazinon achieved high decomposition rate, this depict its biodegradability, this supported by the results of kookana et al.,(1994) which elucidated some pesticides that are more readily biodegradable such as organophosphate. Previous study results for diazinon reported that bacteria Staphylococcus achieved the highest decomposition with concentration level of 50ppm resulted in decomposition of up to 68.66%, this result is different from the results obtained by Tamer Mohamed *et al.*, (2013) which shows non-significant effect on bacterial diazinon degradation, and that bacteria pseudomonas and bacillus showed the ability to degrade diazinon insecticides more than the others.

For the pesticide pirimicarb we found that it has achieved a high rate of biodegradation. It is very close to the chemical properties and the toxic act of the pesticide diazinon.

In general, if one considers the differences in microbe physiological properties and its ability to metabolize many substances, it uses different pesticides as its food, which it represents in two ways. First, the chemical supports the growth of microorganisms where they are used as a source of carbon and energy as happened to pirimicarb and diazinon, and sometimes as a source of nitrogen like atrazine, this is consistent with the report by Mandelbaum *et al.*, (1995). In this case; the density of the number of bacteria and disappearance or lack of chemical compound is predominant.

The second method in which the chemical is not used as a food source, and use one of the compounds resulting from the decomposition of one of the other bacterial species and this explains the high rate of decay of pesticides when using different mixtures of bacteria.

Diazinon has the highest percentage of decomposition. They also have a mechanism of action on the enzyme acetylcholine esterase ACHE and not affecting the bacteria. This is in consistent with findings by Philip Mwenda (2011). The present subject examines applications and future use of OP-degrading microorganism cultures from agricultural fields for bioremediation.

From these results it can be said that all types of bacteria isolated from agricultural soils proved the ability of the biological decomposition of pesticides under study with different levels of efficiency, these results propose useful information for the potential application of the bacteria strain in bioremediation of pesticide-contaminated environments, which confirms the ability of microorganisms to remove pesticides from contaminated media.

It is also clear from the present study that the pesticide diazinon and pirimicarb are highly susceptible to degradation compared to pesticide atrazine which showed moderate degradation, although Brandon and his coworkers (1997) showed that atrazine biodegradation was higher in liquid cultures than soil.

The bacterial species isolated from soil, especially *S.aureus* showed the ability to degrade pesticides.

E. coli and *Sa. bongori* showed less efficiency in decomposition, but play an important role in biodegradation of the studied pesticides.

Mixing the bacteria with each other increased the their efficiency, especially when S.aureus bacteria was included.

Diazinon and pirimicarb are highly susceptible to degradation, compared to atrazine.

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