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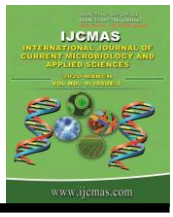

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Effectiveness evaluation of Bacterial Species Isolated from soil in Bioremediation of Diazinon, Pirimicarb and Atrazine Pesticides

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A B S T R A C T

In the present investigation, bacterial species such as *E. coli*, *S. aureus* and *S. bongori* were isolated from soil by using serial dilution. Bioremediation results showed the *S. aureus* was highly efficient on Diazinon removal by 62%, 63.2% and 68.6%, Pirimicarb removal was 44%, 52.4% and 53.8%, and Atrazine removal was 61%, 65.6% and 70.6%. and the efficiency of *E. coli* removal on Diazinon was 59%, 60.8% and 63.8%; on Pirimicarb was 44%, 52.4% and 53.8%; and for Atrazine 57%, 60.8% and 64.4%. *S. bongori* efficiency on Diazinon was 49%, 51.2% and 55.8%; on Pirimicarb removal was 61%, 63.2% and 68.4%; Also, in Atrazine removal 48%, 50.4% and 57.2%. When comparing the growth rate of bacterial cells. The bacterial cells before treatment with *S. aureus* was 22.01×, Results after treatment showed Diazinon of 35.58×. The Pirimicarb 32.41× and Atrazine was 38.45 ×, either *E. coli*. Its bacterial growth was before treatment 17.09×. To show the results of growth on diazinon 30.43×, Pirimicarb 27.71× and Atrazine 24.34×. While the growth was in S.bongori Before treatment 10.09× While recorded a growth rate on Diazinon 18.82×, Pirimicarb 19.98× and Atrazine 17.08 ×. These bacterial species efficiencies on bioremediation of these three pesticides proved to be promising It can be used safely in the process of removing pesticides, yet more research on safety, mechanisms and kinetics needs to be further investigated.

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 isolated bacterial species on bioremediation of pesticides diazinon, pirimicarb and atrazine. Evaluation of the level of pesticide removal by 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**.**

No	Pesticide	Group	Type	Chemical formula
1	diazinon	OP	Insecticide	$C_{12}H_{21}N_2O_3PS$
2	pirimicarb	carbamate	Insecticide	$C_{11}H_{18}N_4O_2$
3	atrazine	triazine	Herbicides	$C_8H_{14}ClN_5$

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:

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

4. Average of cell / square :

$$= \frac{viable\ cell}{squaer}$$

5. Dilution factor:

$$= \frac{final\ volume}{volume\ of\ cell}$$

6. Concentration (viable cell / ml):

=average of cell / square × dilution factor × 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 $MgSO_4$ 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

$$\frac{\text{concentrate solution before treatment} - \text{concentrate solution after treatment}}{\text{concentrate solution before treatment}} \times 100$$

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 isolated bacterial species on bioremediation of pesticides diazinon, pirimicarb and atrazine

The results of the interaction effects between the three studied factors bacteria, pesticides and pesticide concentrations in bioremediation of pesticides was recorded for *S.aureus* against atrazine for 50ppm concentration, followed by *S.aureus* with Diazinon at a concentration level of 50ppm and the *S.aureus* with atrazine at a concentration level of 25ppm while, the lowest value was found in *E. coli* with Pirimicarb at concentration level of 10ppm followed *S.bongori* with Diazinon in concentration 10ppm and *S.bongori* with atrazine at a concentration of 10ppm, which were presented in Table (2). From the previous results, we found that, there are significant differences between the types of bacteria in bioremediation efficiency Table (3). The *S. aureus* bacteria was the most effective species (63.99%). *E.coli* bacteria had an average efficiency of 58.21%. Finally,

S.bongori bacteria are considered to be effective as low as 56.63%.

Evaluation of the level of pesticide removal by bacterial species

In the treatment of pesticides with each bacterium separately, it was found that Diazinon and Pirimicarb scored the highest value compared to Atrazine Table (4).

Comparison of growth rate of bacterial cells in pesticides

When comparing the growth rate of bacterial cells. The bacterial cells before treatment with *S. aureus* was 22.01×, Results after treatment showed diazinon of 35.58×. The Pirimicarb 32.41× and atrazine was 38.45 × (Fig. 1) Either E. coli Its bacterial growth was before treatment 17.09× To show the results of growth on diazinon 30.43×, Pirimicarb 27.71× and atrazine 24.34 × (Fig. 2) While the growth was in *S. bongori*. Before treatment 10.09× While recorded a growth rate on diazinon 18.82× , Pirimicarb 19.98× and atrazine 17.08 × (Fig. 3).

Reported results showed clearly that, the bacterium *S.aureus* have the highest capacity and efficiency in bioremediation processes, showing proportion of analytical efficiency of 63.99% at concentration levels of the three pesticides. Then followed by bacteria *E. coli* with a medium efficiency is achieved for a percentage of 58.21%. This result is consistent with (Radhika and Kannahi, 2014). They reported that *S.aureus* and *E. coli* in bioremediation of Permethrin. The *S.bongori* bacteria have shown the lowest level of efficiency of 56.36%, with a slight difference from *E. coli*. It is possible to say that these results are consistent with many previous studies that show the ability of microorganisms such as fungi and bacteria to consume a wide range of pesticides. In most cases the ability of microorganisms to consume one or more compounds as a source of energy and carbon (Alzawy *et al.*, 2013). It is also observed when comparing the growth of bacterial cells to the species used in bioremediation. The superiority of *S.aureus* bacteria was observed as the growth rate of bacterial cells at the concentration levels of Diazinon, Pirimicarb and Atrazine where it was 38.01×, 35.36× and 33.43× Respectively, as well as bacteria E. coli 32.22×, 30.43×and 31.43*. Either bacteria *S. bongori* which is less efficient and also the least growth rate 21.58×, 22.89× and 20.71×respectively.

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

No	Tests	*S.bongori*	*E.coli*	*S.aureus*
1	Indole	-	+	-
2	Methyl Red (MR)	+	+	+
3	Urease test	-	-	+
4	Catalase	+	+	+
5	Motility	+	+	-
6	Citrate	+	-	-
7	Gram test	-	-	+

Table.2 Effect of interaction between bacteria, pesticides and pesticide concentrations on the ratio of biological treatment

Bacteria	Pesticides	Con / ppm	Result
	Diazinon	10	59.03 d-g
		25	60.76 c-e
		50	63.63 a-e
	Pirimicarb	10	44.33 k
		25	52.83 f-j
E. coli		50	60.93 b-e
	Atrazine	10	57.30 e-i
		25	60.63 c-f
		50	64.46 a-e
	Diazinon	10	62.33 b-e
		25	63.40 a-e
		50	68.66 a-b
	Pirimicarb	10	59.33 c-g
		25	61.53 b-e
S. aureus		50	64.50 a-e
	Atrazine	10	60.33 c-f
		25	65.26a-d
		50	70.53 a
	Diazinon	10	49.56 i-k
		25	51.73 g-k
		50	58.33 d-g
	Pirimicarb	10	61.33 b-e
		25	63.50 a-e
S. bongori		50	66.96 a-c
	Atrazine	10	48.26 j-k
		25	50.13 h-k
		50	57.43 e-h

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

Table.3 Comparison of the efficiency of single bacteria in bioremediation of pesticides

Bacteria	**Result**
E.coli	58.21 b
S. aureus	63.99 a
S. bongori	56.36 c

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

Table.4 Compared to the efficiency of the decomposition of pesticides with individual bacteria

Pesticides	Result
Diazinon	62.73 a
Pirimicarb	64.07 a'b
Atrazine	58.67 b

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

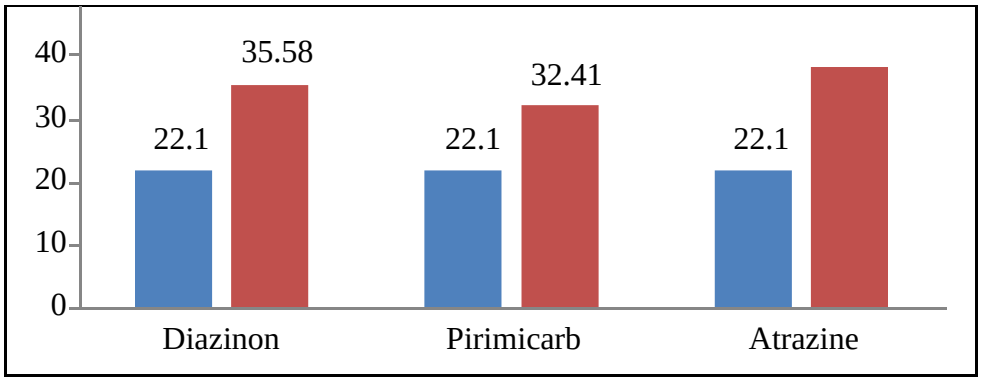

Figure.1 *S.aureus* growth rate

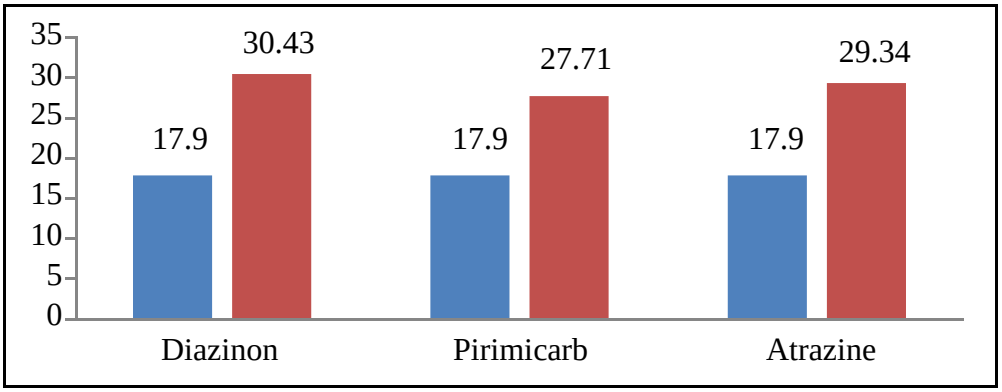

Figure.2 *E. coli* growth rate

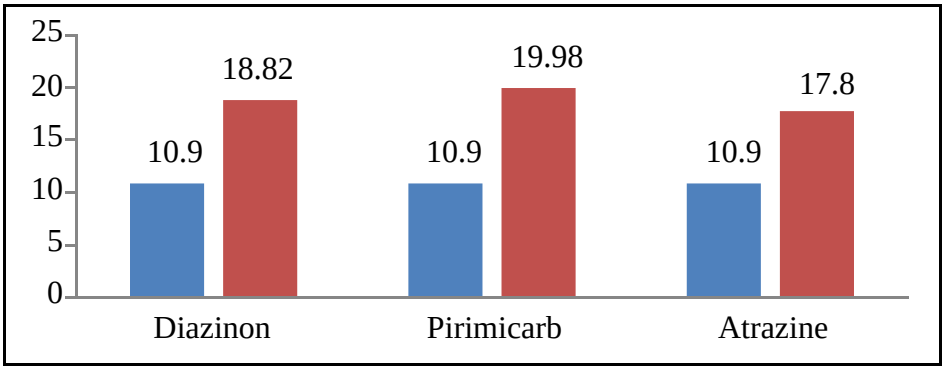

Figure.3 *S.bongori* growth rate

This indicates that bacteria are unable to take advantage of the carbon and energy present in the pesticide to help them in this growth and reproduction process, thus facilitating the growth of bacterial cells. In the end, the overall result is either the elimination of the pesticide or its conversion to other compounds as a result of metabolism. The above findings indicate that there are clear general differences combination mixture of bacteria and pesticides, but at different scales, with regard to the overall trend of pesticide degradation. In the results of the analysis of pesticides with the single treatment of bacteria; it is found that, the pesticide Atrazine have the lowest rate of decomposition of 58.67%, compared to pesticides Pirimicarb 64.07% and Diazinon 62.73%, although there were no significant differences between them through statistical analysis, however, they gave the highest proportion of decomposition. 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 Pirimicarb 71.65%. 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.*,1995), who concluded on others pesticides such as Atrazine and Simazine are biodegradable at slow rates and may by leached from soil to ground water .Conversely, we found that pesticide Diazinon achieved high decomposition rate, this depict its biodegradability, this supported by the results of (Kookana *et al.*, 1995), which elucidated some pesticides that are more readily biodegradable such as organophosphate. Previous 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 difference in microbiology in its 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 (Mandelbaum *et al.,* 1995). In this case; the density of the number of bacteria and disappearance or lack of chemical compound is predominant.

In conclusion, the bacterial species isolated from soil especially *Staphylococcus aureus* showed the ability to degrade pesticides. Bacteria *E.coli* and *salmonalla bongori* showed less efficiency in decomposition but could benefit from them. Diazinon and Pirimicarb are highly susceptible to degradation compared to pesticide Atrazine.

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