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Biological Degradation of Metribuzin and Profenofos by some Efficient Bacterial Isolates

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ABSTRACT

The soil sample was collected from the paddy field of Sriperumbudur, Tamilnadu which is having a history of repeated pesticide applications. The isolation of efficient pesticide degrading bacteria was identified as *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Bacillus subtilis*. The growth of the three pesticide degrading isolates was assessed in Minimal salt broth containing 25 ppm of pesticides. Two popularly used pesticides Metribuzin and Profenofos were selected for this study. Among the three bacterial isolates, the bacteria *Bacillus subtilis* utilized the pesticides effectively and showed maximum growth. The growth of the three pesticides degrading isolates *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Bacillus subtilis* was assessed in Minimal salt broth containing 25 ppm of pesticides at different temperature levels (25 °C, 30 °C, 35 °C, 40 °C, 45 °C, 50 °C & 55 °C) and pH levels (pH 4, pH 5, pH 6, pH 7 & pH 8) and carbon sources *(*Lactose, Dextrose, Fructose, Mannose & Galactose) and nitrogen sources Peptone, Yeast extract, Beef extract, Malt extract and Casein respectively. The maximum growth rate of bacteria was recorded at 35 °C and pH 6. The maximum growth of bacteria was in the presence of Dextrose followed by Fructose, Galactose and Mannose. The least growth was recorded in Lactose broth culture. The maximum growth of bacteria was in the presence of Malt extract followed by Peptone, Yeast extract and Casein. The least growth was recorded in Beef extract broth culture. The bacterial isolates showed maximum growth in the Minimal salt broth containing Profenofos followed by Metribuzin.

***Keywords*:** Metribuzin; Profenofos; *Pseudomonas aeruginosa*; *Staphylococcus aureus*; *Bacillus subtilis*

1. INTRODUCTION

India is an agriculture based country. About 60-70 % of its population is dependent on agriculture. A major portion of arable land already under cultivation is being rapidly depleted by industries and urban encroachments. On the other hand, the demand for agricultural crops is increasing day by day due to the rapidly increasing population. Hence, there is a need for a huge increase in the quantity and quality of agricultural produce. To meet these objectives; agrochemicals like insecticides, fungicides, pesticides, and herbicides and also; use of better

quality seeds are being used on a large scale in agricultural lands. About 30 % of agricultural produce is lost due to pests.

Hence the use of pesticides becomes indispensable in agriculture [1]. At present more than 250 active substances and approximately 10,000 formulations have been incorporated in India and which are known as pesticides. Insecticides represent the greatest proportion of pesticides used in developing countries, whereas herbicide sales have been greater than those of other pesticides in industrialized countries. Now a day Biopesticides are also great importance in India.

Although some persistence organochlorine pesticides have been banned from agricultural and public health use during the past few decades, high concentrations of pesticides and its metabolites have been found in soil, water, and sediment samples [2-4]. Furthermore, other insecticides, such as endosulfan and lindane, are currently restrict in use throughout the world [5] and their presence in air, water, and soil is a problem of great concern. Microbial degradation of pesticides applied to soil is the principle mechanism which prevents the accumulation of these chemicals in the environment to reducing their levels in the environment has therefore become an important goal.

2. MATERIALS AND METHODS

2. 1. Collection of soil sample

The soil samples were collected from the different places of paddy fields of Sriperumbudur, Tamilnadu which is having a history of repeated pesticide applications. The collected soil samples were ground, passed through 2 mm sieve and stored at 4 °C.

2. 2. Pesticides used

Pesticides used in this present study are

a) Profenofos
b) Metribuzin

2. 3. Isolation and identification of bacterial isolates

Pour plate technique was used for the isolation of efficient pesticide degrading bacteria in Nutrient agar and King,s B agar plate. Well grown bacterial colonies were picked and further purified by streaking method. The isolated strains were maintained on Nutrient agar and King,s B agar slants and stored at 4 °C. Identification of the three different bacterial isolates was carried out by the routine bacteriological methods i.e., By the colony morphology, preliminary tests like Gram staining, Capsule staining, Endospore staining, Motility, Catalase and Oxidase, Plating on selective media and by performing biochemical tests.

2. 4. Determination of the growth of pesticide degrading bacterial isolates on minimal salt broth

The suspension of 24 hours old cultures of *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Bacillus subtilis* were used to prepare the bacterial inoculum. They were prepared in saline solution (0.85 % sodium chloride). A loopful of *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Bacillus subtilis* cultures were inoculated into 25 ml of saline and incubated at 37 °C for 3 hours.

The growth of pesticide degrading bacterial isolates (*Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Bacillus subtilis*) was determined by using Minimal Salt Broth. For this, 2 ml of the bacterial inoculum was inoculated into 100 ml of Mineral salt broth containing 25 ppm of two different pesticides. The flasks were then incubated at 37 °C for 7 days in a microbial shaker at 150 rpm. Five ml of culture was drawn and centrifuged at 5000 rpm for 10 minutes. The pellet was discarded and the supernatant was collected to evaluate the growth of pesticide degrading bacteria. The growth of the pesticide degrading bacterial isolates was assessed by using UV – spectrophotometer at 542 nm.

2. 5. Effect of temperature for the growth of pesticide degrading bacterial isolates

To study the stability of the bacterial isolates for the biodegradation of pesticides, an experiment conducted in a Erlenmeyer flask containing 25 ppm of pesticides in 100 ml Minimal salt broth. After sterilization by autoclaving the flask were cooled and inoculated with the bacterial cultures and maintained at different temperature (25 °C, 30 °C, 35 °C, 40 °C, 45 °C, 50 °C and 55 °C). After 24 hours, 10 ml of culture was drawn and centrifuged at 5000 rpm for 10 minutes and the pellet were discarded and the supernatant was collected to evaluate the growth of pesticide degrading bacteria. The optical density was taken at 542 nm using UV – spectrophotometer.

2. 6. Effect of pH for the growth of pesticide degrading bacterial isolates

To study the stability of the bacterial isolates for the biodegradation of pesticides, an experiment conducted in a Erlenmeyer flask containing 25 ppm of pesticides in 100 ml Minimal salt broth. After sterilization by autoclaving the flask were cooled and inoculated with the bacterial cultures and maintained at different pH (4, 5, 6, 7 and 8). After 24 hours, 10 ml of culture was drawn and centrifuged at 5000 rpm for 10 minutes. The pellet was discarded and the supernatant was collected to evaluate the growth of pesticide degrading bacteria and the optical density was taken at 542 nm using UV – spectrophotometer.

2. 7. Effect of carbon sources for the growth of pesticide degrading bacterial isolates

The effect of various carbon sources for the maximization of pesticide biodegradation was tested. The bacterial isolates were cultivated in 100 ml of Mineral salt broth with 25 ppm of pesticides and 1 g of various carbon sources (Lactose, Dextrose, Fructose, Mannose and Galactose) and incubated at 37 °C. After 24 hours, 10 ml of culture was drawn and centrifuged at 5000 rpm for 10 minutes. The pellet was discarded and the supernatant was collected to evaluate the growth of pesticide degrading bacteria and the optical density was taken at 542 nm using UV – spectrophotometer.

2. 8. Effect of nitrogen sources for the growth of pesticide degrading bacterial isolates

The effect of various carbon sources for the maximization of pesticide biodegradation was tested. The bacterial isolates were cultivated in 100 ml Minimal salt broth with 25 ppm of pesticides and 1 g of various nitrogen sources (Peptone, Yeast extract, Beef extract, Malt extract and Casein) and incubated at 37 °C. After 24 hours, 10 ml of culture was drawn and centrifuged at 5000 rpm for 10 minutes. The pellet was discarded and the supernatant was collected to evaluate the growth of pesticide degrading bacteria and the optical density was taken at 542 nm using UV – spectrophotometer.

3. RESULTS AND DISCUSSION

In this present experiment, the growth of the three pesticide degrading isolates *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Bacillus subtilis* was assessed in Minimal salt broth containing 25 ppm of pesticides. Two different pesticides Metribuzin and Profenofos were used in this study. Among the three bacterial isolates, the bacteria *Bacillus subtilis* utilized the pesticides effectively and showed maximum growth (OD value) followed by *Staphylococcus aureus and Pseudomonas aeruginosa.* The bacterial isolates showed (Table and Fig 1 & 2) maximum growth in the Minimal salt broth containing Profenofos (0.586) followed by Metribuzin (0.462).

Table 1. Growth of pesticide degrading bacterial isolates on Minimal Salt broth containing Metribuzin.

Days	*Pseudomonas aeruginosa* (OD at 542 nm)	*Staphylococcus aureus* (OD at 542 nm)	*Bacillus subtilis* (OD at 542 nm)
0	0.213	0.239	0.268
1	0.258	0.284	0.372
2	0.274	0.317	0.349
3	0.301	0.336	0.351
4	0.396	0.369	0.364
5	0.352	0.381	0.405
6	0.375	0.415	0.448
7	0.406	0.453	0.462

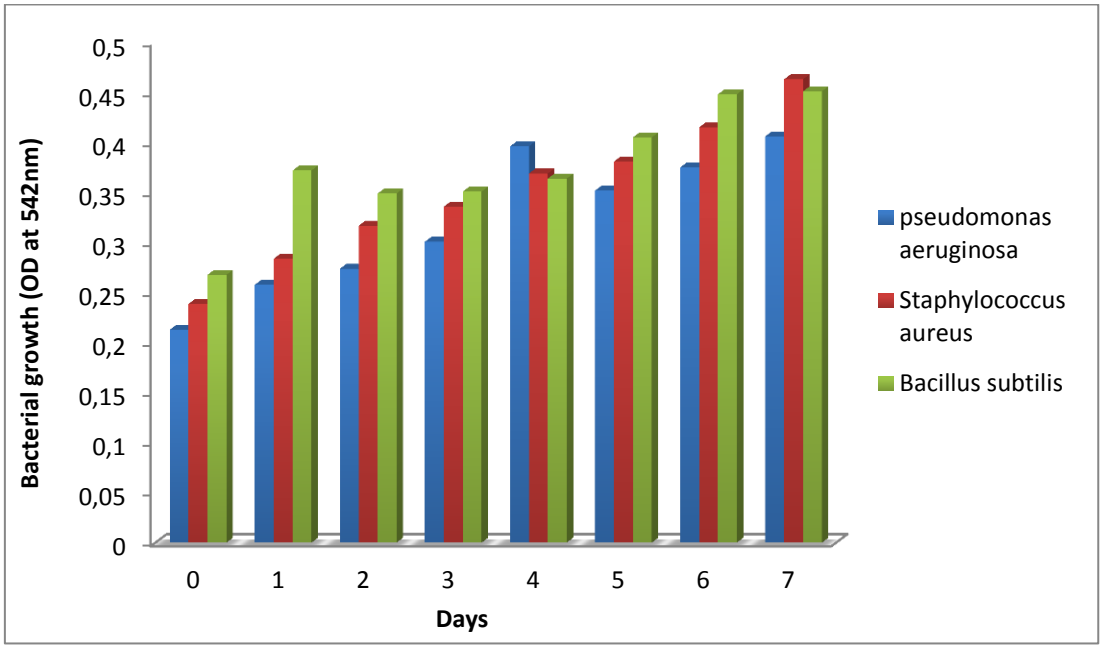

Fig. 1. Clustered column chart of Growth of pesticide degrading bacterial isolates on Minimal Salt broth containing Metribuzin.

Table 2. Growth of pesticide degrading bacterial isolates on Minimal Salt broth containing Profenofos.

Days	*Pseudomonas aeruginosa* (OD at 542 nm)	*Staphylococcus aureus* (OD at 542 nm)	*Bacillus subtilis* (OD at 542 nm)
0	0.295	0.371	0.386
1	0.349	0.409	0.429
2	0.371	0.436	0.461
3	0.398	0.468	0.493
4	0.406	0.482	0.508
5	0.454	0.501	0.514
6	0.487	0.524	0.552
7	0.501	0.547	0.586

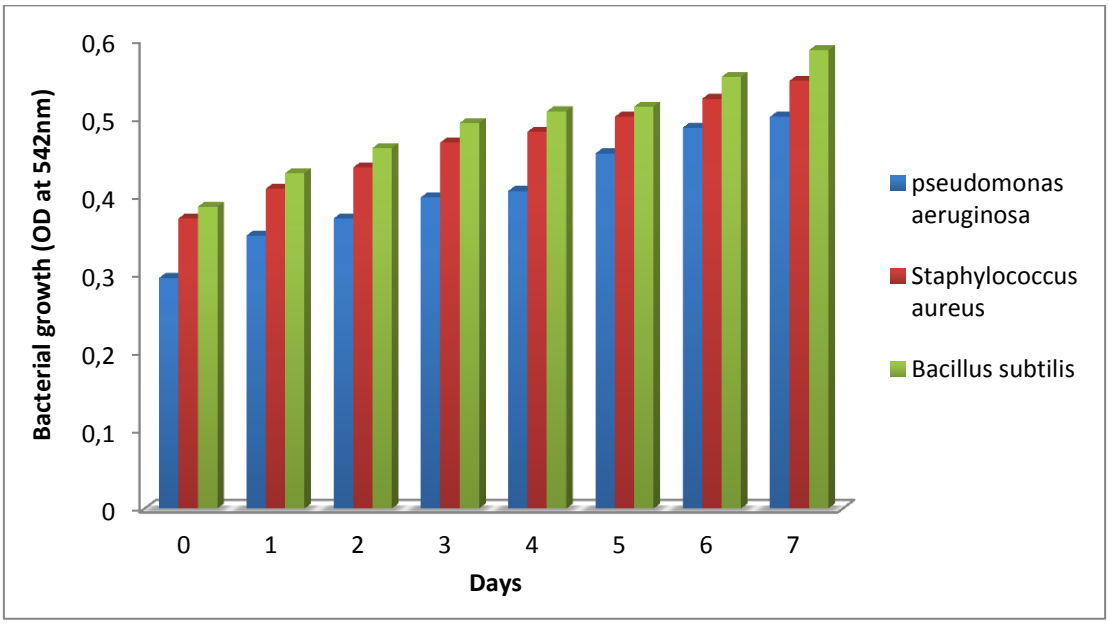

Fig. 2. Clustered column chart of Growth of pesticide degrading bacterial isolates on Minimal Salt broth containing Profenofos.

Microorganisms are involved in soil processes such as recycling of essential plant nutrients, humus formation and soil structure stability. The addition of pesticides may disturb the equilibrium and thus fertility of the soil. The chances of isolating microbial strains from polluted soils, with high ability to metabolize a particular xenobiotic are brighter [6]. During enrichment with a xenobiotic compound, the natural selection of microorganisms which have been adapted to the presence of that xenobiotic and its rapid biodegradation are known to take place [7]. Recently, Murugesan *et al*. [8] studied the ability of five bacterial isolates (*Pseudomonas aeruginosa*, *Bacillus subtilis*, *Escherichia coli* and *Corynebacterium*) to degrade cypermethrin. It was confirmed that these isolated organisms were able to utilize and degrade cypermethrin. On that five different bacterial colonies, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Escherichia coli* were found active in utilizing cypermethrin (1 %) where as

Bacillus subtilis and *Corynebacterium* were moderately active in utilizing cypermethrin (0.1 %). The growth curve experiment was performed at 0.1 and 1 % dose of cypermethrin to analyze the viable count of *Pseudomonas aeruginosa*.

In this research, the growth of the three pesticides degrading isolates *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Bacillus subtilis* was assessed in Minimal salt broth containing 25 ppm of pesticides at different temperature levels 25 °C, 30 °C, 35 °C, 40 °C, 45 °C, 50° C and 55 °C. Two different pesticides Metribuzin and Profenofos were used in this study. The maximum growth rate of bacteria was recorded at 35 °C followed by 30 °C, 25 °C, 40 °C, 45 °C and 50 °C. The least growth rate was recorded at 55 °C. Among the three bacterial isolates, the bacteria *Bacillus subtilis* utilized the pesticides effectively and showed (Table and Fig. 3 & Fig. 4) maximum growth followed by *Pseudomonas aeruginosa* and *Staphylococcus aureus*. The bacterial isolates showed maximum growth in the Minimal salt broth containing Profenofos (0.789) followed by Metribuzin (0.675).

Table 3. Effect of temperature for the growth of pesticide degrading bacteria in Minimal Salt broth containing Metribuzin.

Temperature °C	*Pseudomonas aeruginosa* (OD at 542nm)	*Staphylococcus aureus* (OD at 542 nm)	*Bacillus subtilis* (OD at 542 nm)
25	0.493	0.581	0.626
30	0.528	0.615	0.643
35	0.597	0.652	0.675
40	0.504	0.549	0.584
45	0.399	0.453	0.492
50	0.361	0.407	0.469
55	0.326	0.388	0.431

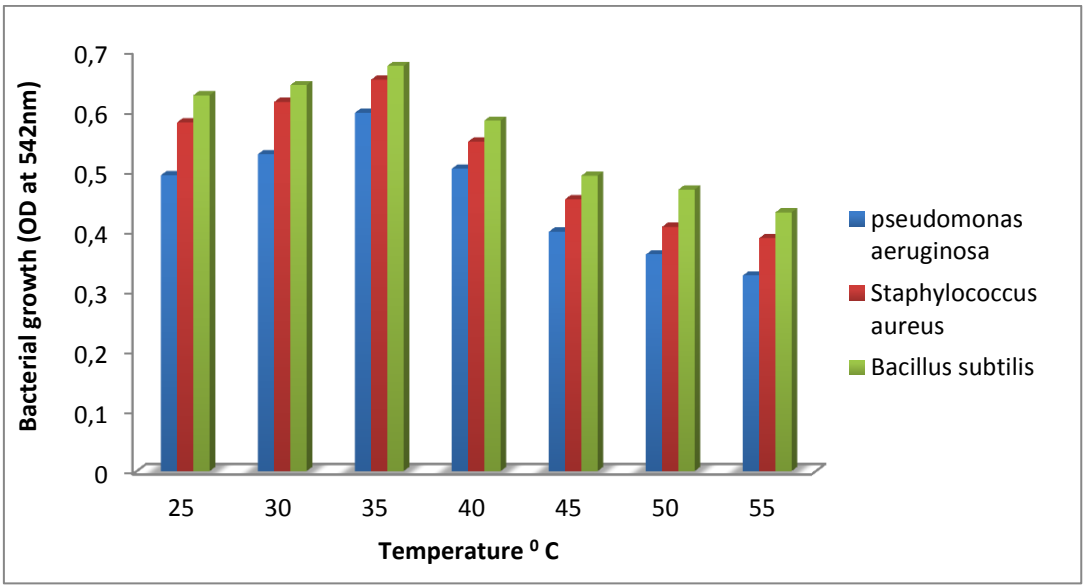

Fig. 3. Clustered column chart of temperature for the growth of pesticide degrading bacteria in Minimal Salt broth containing Metribuzin.

Table 4. Effect of temperature for the growth of pesticide degrading bacteria in Minimal Salt broth containing Profenofos.

Temperature °C	*Pseudomonas aeruginosa* (OD at 542 nm)	*Staphylococcus aureus* (OD at 542 nm)	*Bacillus subtilis* (OD at 542 nm)
25	0.587	0.714	0.736
30	0.632	0.739	0.761
35	0.706	0.765	0.789
40	0.614	0.628	0.645
45	0.522	0.593	0.607
50	0.475	0.521	0.568
55	0.428	0.497	0.526

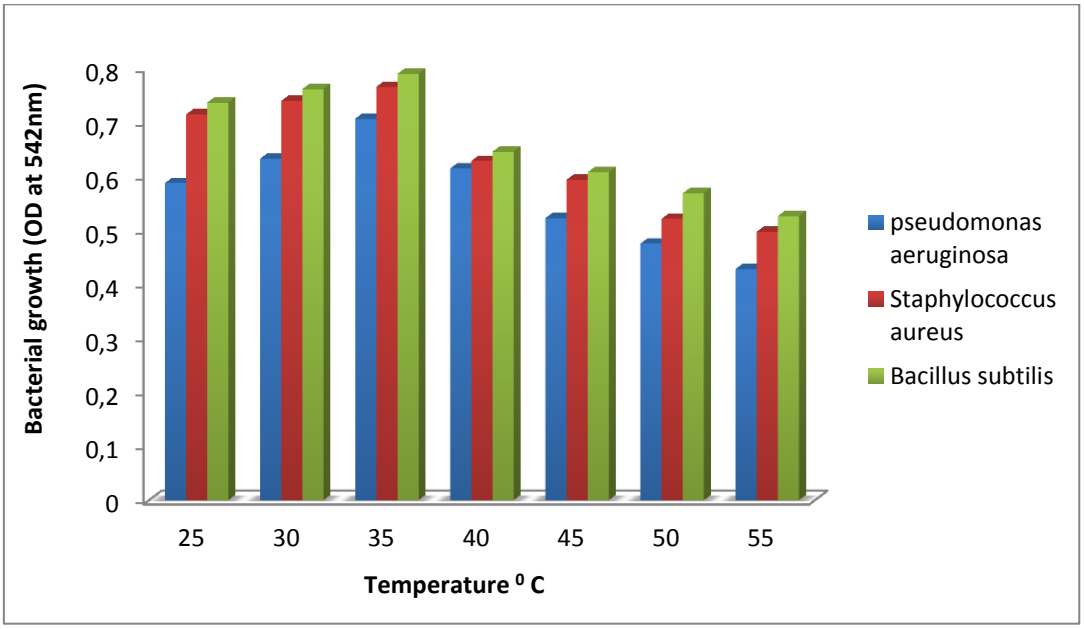

Fig. 4. Clustered column chart of temperature for the growth of pesticide degrading bacteria in Minimal Salt broth containing Profenofos.

The present study well with Perclich and Lockwood [9] observed that incidence of pesticide utilizing bacterial genera such as *Bacillus*, M*icrococcus*, *Pseudomonas* and *Vibrio* in the water and sediment samples of irrigational channel. Walker *et al*. [10] investigated that, Pesticide is mainly degraded by *Pseudomonas* and *Bacillus* and this versatility might be due to the presence of wide range of enzymes.

In this study, the growth of the three pesticides degrading isolates *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Bacillus subtilis* was assessed in Minimal salt broth containing 25 ppm of pesticides at different pH levels pH 4, pH 5, pH 6, pH 7 and pH 8. Two different pesticides Metribuzin and Profenofos were used in this study. The maximum growth rate of bacteria was recorded at pH 6 followed by pH 7, pH 8 and pH 5. The least growth rate of *Bacillus subtilis* was recorded at pH 4. Among the three bacterial isolates, the bacteria

Bacillus subtilis utilized the pesticides effectively and showed maximum growth followed by *Pseudomonas aeruginosa* and *Staphylococcus aureus*. The bacterial isolates showed (Table and Fig. 5 & Fig. 6) maximum growth in the Minimal salt broth containing Profenofos (0.781) followed by Metribuzin (0.669).

Table 5. Effect of pH for the growth of pesticide degrading bacteria in Minimal Salt broth containing Metribuzin.

pH	*Pseudomonas aeruginosa* (OD at 542 nm)	*Staphylococcus aureus* (OD at 542 nm)	*Bacillus subtilis* (OD at 542 nm)
4	0.376	0.442	0.497
5	0.532	0.568	0.611
6	0.581	0.594	0.669
7	0.559	0.586	0.538
8	0.543	0.572	0.524

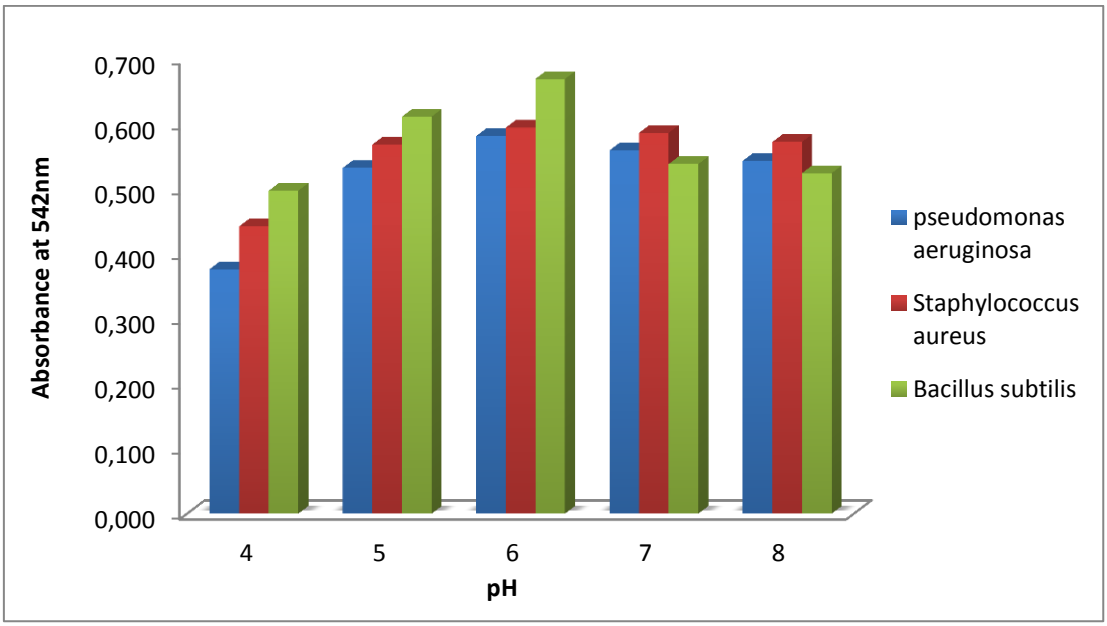

Fig. 5. Clustered column chart of pH for the growth of pesticide degrading bacteria in Minimal Salt broth containing Metribuzin.

Enhanced degradation of Profenofos by *Enterobacter* strain was reported by [11]. Yang *et al*. [12] isolated *Alkaligenes faecalis*, which is capable of degrading Profenofos and 3, 5, 6-trichloro-2-pyridinol (TCP). Six Profenofos-degrading bacteria were isolated using Profenofos as the sole carbon source by an enrichment procedure [13].

Table 6. Effect of pH for the growth of pesticide degrading bacteria in Minimal Salt broth containing Profenofos.

pH	*Pseudomonas aeruginosa* (OD at 542 nm)	*Staphylococcus aureus* (OD at 542 nm)	*Bacillus subtilis* (OD at 542 nm)
4	0.481	0.558	0.593
5	0.626	0.685	0.725
6	0.769	0.723	0.781
7	0.694	0.689	0.764
8	0.652	0.647	0.752

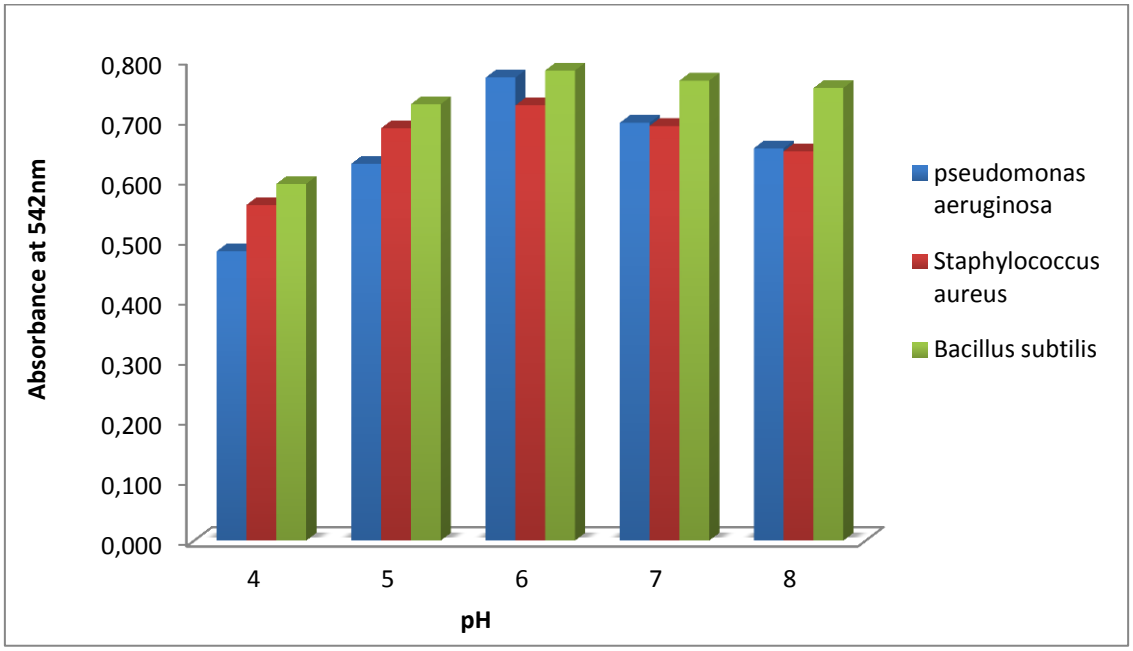

Fig. 6. Clustered column chart of pH for the growth of pesticide degrading bacteria in Minimal Salt broth containing Profenofos.

Choi *et al.* [14] isolated three parathion-degrading bacteria and eight pairs of bacteria showing syntrophic metabolism of parathion from rice field soils, and investigated their genetic and phenotypic characteristics. The three isolates and eight syntrophic pairs were able to utilize parathion as a sole source of carbon and energy, producing p-nitrophenol as the intermediate metabolite during the complete degradation of parathion.

In the present study, the growth of the three pesticides degrading isolates *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Bacillus subtilis* was assessed in Minimal salt broth containing 25 ppm of pesticides using different carbon sources Lactose, Dextrose, Fructose, Mannose and Galactose. Two different pesticides Metribuzin and Profenofos were used in this study. The maximum growth of bacteria was in the presence of Dextrose followed by Fructose, Galactose and Mannose.

The least growth was recorded in Lactose. Among the three bacterial isolates, the bacteria *Bacillus subtilis* utilized the pesticides effectively and showed maximum growth followed by *Staphylococcus aureus and Pseudomonas aeruginosa*. The bacterial isolates showed (Tabe and Fig. 7 & Fig. 8) maximum growth in the Minimal salt broth containing Profenofos (0.784) followed by Metribuzin (0.686).

Table 7. Effect of carbon sources for the growth of pesticide degrading bacteria in Minimal Salt broth containing Metribuzin.

Carbon source	*Pseudomonas aeruginosa* (OD at 542 nm)	*Staphylococcus aureus* (OD at 542 nm)	*Bacillus subtilis* (OD at 542 nm)
lactose	0.491	0.522	0.596
Dextrose	0.624	0.647	0.686
Fructose	0.548	0.603	0.668
Mannose	0.511	0.588	0.562
Galactose	0.569	0.601	0.573

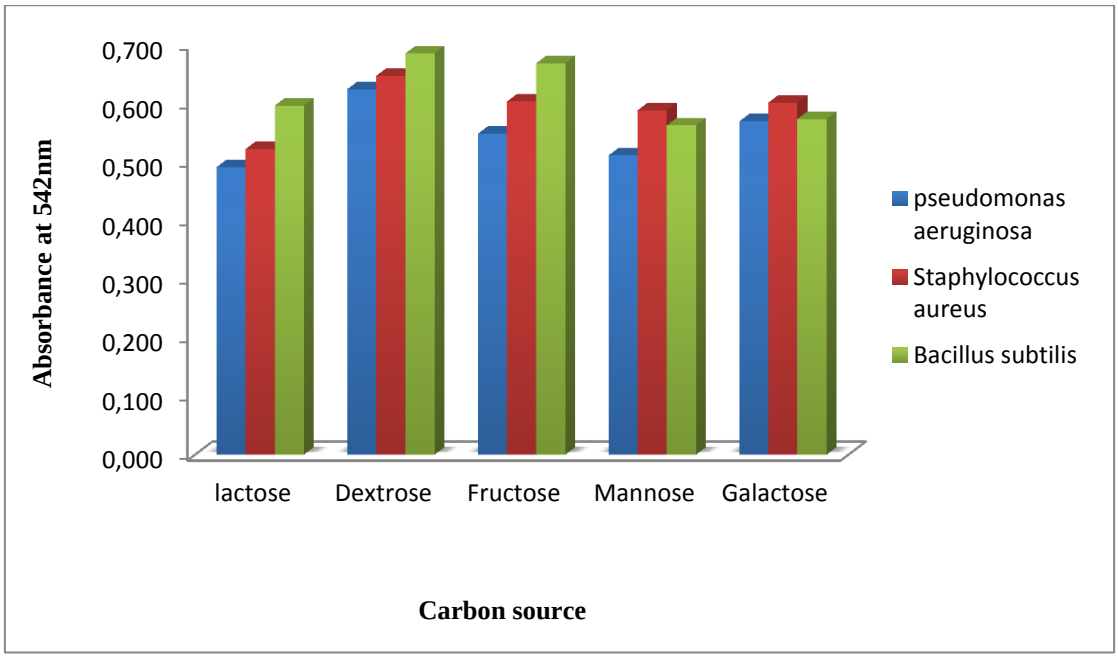

Fig. 7. Clustered column chart of carbon sources for the growth of pesticide degrading bacteria in Minimal Salt broth containing Metribuzin.

Table 8. Effect of carbon sources for the growth of pesticide degrading bacteria in Minimal Salt broth containing Profenofos.

Carbon source	*Pseudomonas aeruginosa* (OD at 542 nm)	*Staphylococcus aureus* (OD at 542 nm)	*Bacillus subtilis* (OD at 542 nm)
lactose	0.597	0.644	0.699
Dextrose	0.723	0.756	0.784
Fructose	0.669	0.723	0.765
Mannose	0.635	0.704	0.701
Galactose	0.681	0.716	0.738

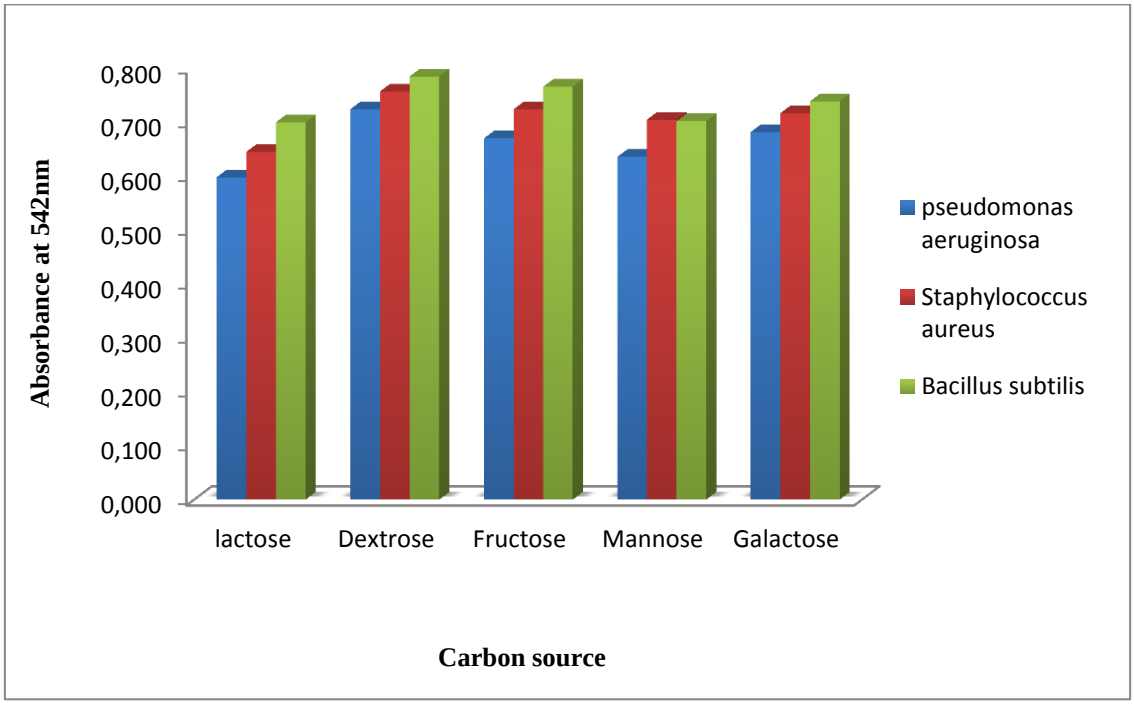

Fig. 8. Clustered column chart of carbon sources for the growth of pesticide degrading bacteria in Minimal Salt broth containing Profenofos.

In this present research, the growth of the three pesticide degrading isolates *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Bacillus subtilis* was assessed in minimal salt broth containing 25 ppm of pesticides using different nitrogen sources Peptone, Yeast extract, Beef extract, Malt extract and Casein. Two different pesticides Metribuzin and Profenofos were used in this study.

The maximum growth of bacteria was in the presence of Malt extract followed by Peptone, Yeast extract and Casein. The least growth was recorded in Beef extract. Among the three bacterial isolates, the bacteria *Bacillus subtilis* utilized the pesticides effectively and showed maximum growth followed by *Staphylococcus aureus and Pseudomonas aeruginosa*.

The bacterial isolates showed (Table and Fig. 9 & Fig. 10) maximum growth in the Minimal salt broth containing Profenofos (0.771) followed by Metribuzin (0.644).

The result of the study well confirmed by Murugesan *et al*. [8] studied the ability of four bacterial isolates (*Pseudomonas aeruginosa*, *Bacillus subtilis*, *Escherichia coli* and *Corynebacterium*) to degrade cypermethrin.

It was confirmed that these isolated organisms were able to utilize and degrade cypermethrin.

Table 9. Effect of nitrogen sources for the growth of pesticide degrading bacteria in Minimal Salt broth containing Metribuzin.

Nitrogen source	*Pseudomonas aeruginosa* (OD at 542 nm)	*Staphylococcus aureus* (OD at 542 nm)	*Bacillus subtilis* (OD at 542 nm)
Peptone	0.538	0.591	0.632
Yeast extract	0.502	0.566	0.603
Beef extract	0.485	0.531	0.578
Malt extract	0.547	0.595	0.644
casein	0.498	0.553	0.589

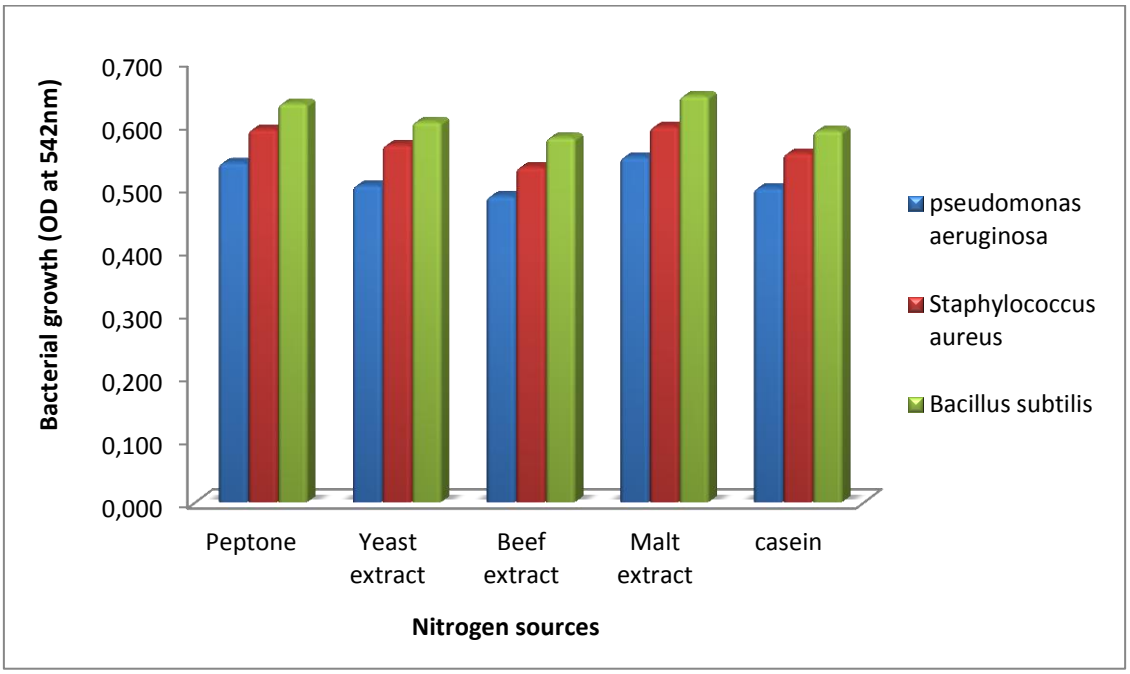

Fig. 9. Clustered column chart of nitrogen sources for the growth of pesticide degrading bacteria in Minimal Salt broth containing Metribuzin.

Table 10. Effect of nitrogen sources for the growth of pesticide degrading bacteria in Minimal Salt broth containing Profenofos.

Nitrogen source	*Pseudomonas aeruginosa* (OD at 542 nm)	*Staphylococcus aureus* (OD at 542 nm)	*Bacillus subtilis* (OD at 542 nm)
Peptone	0.642	0.686	0.757
Yeast extract	0.618	0.671	0.714
Beef extract	0.597	0.643	0.681
Malt extract	0.656	0.695	0.771
casein	0.593	0.661	0.690

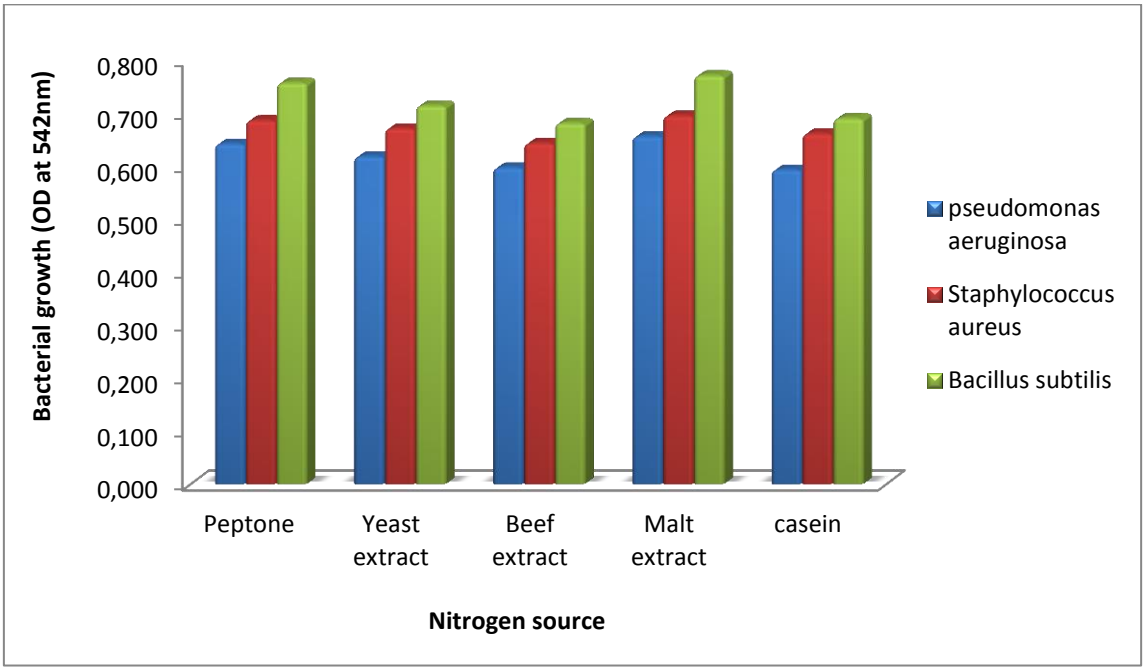

Fig. 10. Clustered column chart of nitrogen sources for the growth of pesticide degrading bacteria in Minimal Salt broth containing Profenofos.

4. CONCLUSIONS

From this study, it was concluded that the bacterial isolates like *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Bacillus subtilis* have the capacity to utilize the pesticides and grow well in the medium supplemented with pesticides as a carbon source. Among the isolated bacteria *Bacillus subtilis* grows well in the presence of pesticides

followed by *Staphylococcus aureus and Pseudomonas aeruginosa*. The isolates *Pseudomonas aeruginosa* and *Staphylococcus aureus* also have the capacity to control many diseases. These bacterial isolates have the capacity to utilize the pesticides as a nutrient and also degrade very fast in the culture broth. It is concluded that both pesticide are biological degradable. Thus use of the both bacterial isolates in the biological treatment of pesticide contaminated soil will give fruitful results. Replacement of chemical pesticides with biopesticides like *Pseudomonas aeruginosa* and *Staphylococcus aureus* will minimize the pesticide contamination in agricultural soil.

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